F1 of 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of
General Medical Sciences
Bethesda, Maryland 20892-6200

http://www.nigms.nih.gov

July 16, 2001

Lawrence Casson
Kenyon and Kenyon
One Broadway
New York, NY 10004

RE: FOI Case No. 26318

Dear Mr. Lawrence Casson:

This is our final response to the February 14 Freedom of Information Act (FOIA) request submitted by Jason Ware who requested copies of grant application, 1R01 GM41478-01A1, -02 and –03. This application was awarded to George P. Smith, Ph.D. by the National Institute of General Medical Sciences of the National Institutes of Health. You asked that the request for –07 be withdrawn. Also, as requested by Mr. Ware, I am sending this material to you since he is no longer handling this matter. Enclosed is a copy of the grant applications.

It is Department of Health and Human Services policy to expunge social security numbers, EIN numbers, birth dates, percentage of effort, institutional base salary, source of private support, pending support, source of private support, references to unpublished articles, and any patentable or proprietary material wherever they appear throughout the grant material. This information has been removed in the enclosed material.

Requesters who ask for grant applications usually want to receive only material that will help in understanding the process that led to the awards, or to improve their own methods of drafting grant applications. Requesters usually do not want material that applicants believe would harm them if released. We have found that the spirit of the FOIA can be enhanced through a spirit of cooperation among requesters of materials and those who submitted the materials.

In this instance, we asked the grantee for advice concerning patent rights and other confidential or financial information and the material that we are furnishing reflects that advice. If you feel that materials have been omitted that should have been made available to you, please write to me and I will consult with the NIH Freedom of Information Officer.

Provisions of the FOIA and Department Regulations allow us to recover part of the cost of responding to your request. Because the cost is below the $25 minimum, there is no charge.

Sincerely,

Yvonne Williams

Yvonne G. Williams
Freedom of Information/Privacy Act Coordinator
The National Institute of General Medical Sciences

Enclosure:
69 pages

45 Center Drive, MSC 6200, Bethesda, MD 20892-6200
Room 2AN32, Phone: (301) 594-5135, (Fax): (301) 480-1969

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

5121MZ

NOTICE OF GRANT AWARD

DATE ISSUED: 06/18/89

GRANT NUMBER: ALY
1 R01 GM41478-01A1

TYPE OF AWARD: RESEARCH
AUTHORIZED BY: 42 USC 241 42 CFR 52

TOTAL PROJECT PERIOD:
From 07/01/89 Through 06/30/

AWARDED BY:
NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

Title of Project or Area of Training
FILAMENTOUS FUSION PHAGE

Grantee Organization
CURATORS OF UNIV OF MISSOURI
GRANTS OFFICE
305 JESSE HALL
COLUMBIA, MO 65211

Principal Investigator/Program Director/Awardee
SMITH, GEORGE P PHD
UNIVERSITY OF MISSOURI
TUCKER HALL
COLUMBIA, MO 65211
09

APPROVED BUDGET
FOR BUDGET PERIOD 07/01/89 Through 06/30/90

Salaries and Wages	$ 54,720
Fringe Benefits	12,643
Total Personnel Costs	$ 67,363
Consultant Costs	
Equipment	2,961
Supplies	12,240
Travel - Domestic	1,440
- Foreign	
Patient Care - Inpatient	
- Outpatient	
Alterations and Renovations	
Consortium/Contractual Costs	
Other	4,275
Trainee Stipends	
Trainee Tuition and Fees	
Trainee Travel	
TOTAL DIRECT COSTS	$ 88,279

When PHS Prior Approval is required for rebudgeting, submit requests to Grants Management Official below.

AWARD COMPUTATION

DIRECT COSTS	$ 88,2
INDIRECT COSTS	$* 32,4
TOTAL	$ 120,7
Less Unobligated Balance (Prior Period(s))	$
AMOUNT OF THIS AWARD →	$ 120,7

Base Dollars	x Rate Percentage	= Indirect Costs
85,318	38.00	32,421

SUPPORT RECOMMENDED FOR REMAINDER OF PROJECT PERIOD

Budget Period	Total Direct Costs (Includes Stipends)
02	81,437
03	73,776
04	76,727
05	79,796
06	NONE

**Subject to availability of funds and satisfactory progress.

REMARKS
THIS GRANT IS INCLUDED UNDER THE EXPANDED AUTHORITIES DESCRIBED IN NIH GUIDE FOR GRANTS AND CONTRACTS, VOLUME 17, NO. 34, 10/21/88.
SEE ATTACHMENT FOR ADDITIONAL TERMS AND CONDITIONS.

PROGRAM ADMINISTRATOR: DR. MARION ZATZ 301/496-0334.
#GRANTS MANAGEMENT: LINDA ROBERTS, LUCY CLARKE, MARCIA THIGPEN (301) 496-77

TERMS OF ACCEPTANCE: By acceptance of funds awarded under this grant, the grantee acknowledges that it will comply with terms and conditions in the following: (1) Legislation cited above; Regulations cited above; (3) Special provisions noted above under remarks or attached to this notice; (4) 45 CFR Part 74 or 92, as applicable; (5) PHS Grants Administration Manual; (6) PHS Grant Policy Statement. The above order of precedence shall prevail.

FY—Common Accounting Number	CRS/Entity Identification No.	PHS List No./Object Class Code	Document Number
9-8423535		/41.4A	(08)R1GM41478A

PROGRAM OFFICIAL
David A. Wolff
DAVID A. WOLFF, PH.D.
ACTING ASSOCIATE DIRECTOR FOR
PROGRAM ACTIVITIES
NAT. INST. GEN. MED. SCIENCES

PHS Grants Management Official
Evelyn W. Carlin
EVELYN W. CARLIN
GRANTS MANAGEMENT OFFICER
OFFICE ASSOC. DIRECTOR PROGRAM
ACTIVITIES, NIGMS

PHS 1533
Rev. 7/88 890614 1042

Copies distributed to Principal Investigator, Program Director or Awardee, and Business Office

MC

C12

U.S. Department of Health and Human Services

Certification Regarding

Drug-Free Workplace Requirements

Grantees Other Than Individuals

By signing and/or submitting this application or grant agreement, the grantee is providing the certification set out below.

This certification is required by regulations implementing the Drug-Free Workplace Act of 1988, 45 CFR Part 76, Subpart F. The regulations, published in the January 31, 1989 Federal Register, require certification by grantees that they will maintain a drug-free workplace. The certification set out below is a material representation of fact upon which reliance will be placed when HHS determines to award the grant. False certification or violation of the certification shall be grounds for suspension of payments, suspension or termination of grants, or governmentwide suspension or debarment.

The grantee certifies that it will provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing a drug-free awareness program to inform employees about:
(1) The dangers of drug abuse in the workplace;
(2) The grantee's policy of maintaining a drug-free workplace;
(3) Any available drug counseling, rehabilitation, and employee assistance programs; and,
(4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will:
(1) Abide by the terms of the statement; and,
(2) Notify the employer of any criminal drug statute conviction for a violation occurring in the workplace no later than five days after such conviction;

(e) Notifying the agency within ten days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction;

(f) Taking one of the following actions, within 30 days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted:
(1) Taking appropriate personnel action against such an employee, up to and including termination; or
(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b), (c), (d), (e) and (f).

ACCEPTANCE

Proposal #910355-1

In accepting this grant, I hereby certify that a drug-free workplace will be provided according to the requirements described above.

Grant No. 1 R01 AM41478-01A1

The Curators of the University of Missouri
The University of Missouri - Columbia
Grantee Organization

6/28/89
Date of Signed Certification

Judson D. Sheridan
Vice Provost for Research &
Dean of the Graduate School
Name/Title of Official Signing for Grantee

(314) 882-6311
Telephone No. of Signing Official

Signature of Above Official

ADDITIONAL TERMS AND CONDITIONS

This award is issued subject to the grantee's executing and submitting the attached Drug Free Workplace Certification within 10 days of receipt. The grantee may not draw down any funds under this grant until the *Certification has been executed and submitted.

*Please return the attached Certification to:

Pat Digges
Westwood Building, Room 940
NIGMS, NIH
Bethesda, MD 20892

NOTE: Certification form sent to official signing for grantee organization.

209092

Form Approved through 9/30/91
OMB No. 0925-0001

DEPT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

NAME: SMITH, GEORGE P
APPL NO:1 R01 GM41478-01A1
GRANT APPLIC DUAL:
IRG:ALY

LEAVE BLANK

COUN DATE: 05/89
RCVD:11-01-88

FOLLOW INSTRUCTIONS

TITLE OF PROJECT (Up to 56 spaces)
Filamentous fusion phage

RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT X NO YES (If "YES," state RFA number and/or announcement title)

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR	NEW INVESTIGATOR
3a. NAME (Last, first, middle) Smith, George P.	3b. DEGREE(S) Ph.D.
3c. SOCIAL SECURITY NUMBER	
3d. POSITION TITLE Associate Professor	3e. MAILING ADDRESS (Street, city, state, zip code) Tucker Hall University of Missouri Columbia, MO 65211
3f. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT Division of Biological Sciences	
3g. MAJOR SUBDIVISION Arts and Science	3h. TELEPHONE (Area code, number and extension) (314) 882-3344

4. HUMAN SUBJECTS
4a. X No Yes — Exemption # _____ OR IRB Approval Date _____
4b. Assurance of Compliance # _____

5. VERTEBRATE ANIMALS
5a. X No Yes IACUC Approval Date _____
5b. Animal Welfare Assurance # _____

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD	7. COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD		8. COSTS REQUESTED FOR ENTIRE PROPOSED PROJECT PERIOD	
	7a. Direct Costs	7b. Total Costs	8a. Direct Costs	8b. Total Costs
From: July 1, 1989 Through: June 30, 1994	$ 98,088	$133,637	$ 420,657	$573,232

9. PERFORMANCE SITES (Organizations and addresses)
405-406 Tucker Hall
University of Missouri
Columbia, MO 65211

Proposal No. 90355-L

10. INVENTIONS (Competing continuation application only)
X NO YES OR Previously reported / Not previously reported

11. APPLICANT ORGANIZATION (Name, address, and congressional district)
Curators of the University of Missouri
University of Missouri-Columbia
Grants Office
305 Jesse Hall
Columbia, MO 65211
9th Congressional District

12. TYPE OF ORGANIZATION
X Public, Specify X State
Private Nonprofit
For Profit (General)
For Profit (Small Business)

13. ENTITY IDENTIFICATION NUMBER

14. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT TOWARDS A BIOMEDICAL RESEARCH SUPPORT GRANT
Code 20 Identification Other academic

15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address and telephone number)
Bob Jenkins, Director
Grants Office, 305 Jesse Hall
University of Missouri-Columbia
Columbia, MO 65211
314/882-7569

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, address and telephone number)
John P. McCormick
Associate Vice Provost for Research
Office of Research, 314 Jesse Hall
University of Missouri-Columbia
Columbia, MO 65211 314/882-9500

17. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001).	SIGNATURE OF PERSON NAMED IN 3a (In ink. "Per" signature not acceptable.) George P. Smith	DATE 10/31/88
18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001).	SIGNATURE OF PERSON NAMED IN 16 (In ink. "Per" signature not acceptable.) John P. McCormick	DATE 10/31/88

PHS 398 (Rev. 9/86)

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR Smith, George P.

DESCRIPTION: State the application's long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the experimental design and methods for achieving these goals. Avoid lengthy summaries of past accomplishments and the use of the first person. This abstract should serve as a succinct and accurate description of the proposed work when separated from the application. DO NOT EXCEED THE SPACE PROVIDED.

The essense of fusion phage is that peptides encoded by cloned inserts are displayed on the virion surface. This allows phage displaying a specific target peptide to be affinity-purified from at least a 10^8-fold background of phage bearing other, non-target sequences, and cloned simply by infecting an E. coli host--an attractive alternative to immunoscreening of conventional expression vectors like lambda gt11. I propose to construct an "epitope library", consisting of 10^6 fusion phage clones displaying random hexapeptides encoded by degenerate oligonucleotide inserts. Since epitopes are typically about 6 amino acids long, and since most hexapeptides are represented, the library may encompass clones reactive with most anti-protein antibodies. I also hope to construct a library of fusion phage displaying cloned antibodies with a vast array of different antigen-binding specificities, so that clones encoding antibodies of defined specificity can be affinity-purified with antigen. This way of obtaining monoclonal antibodies would be much easier than present methods, which require minipulation of animals and cultured cells.

PERSONNEL	NAME	ROLE	ORGANIZATION
	Smith, George P.	Associate Professor--P.I.	Division of Biological Sciences, University of Missouri-Columbia

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Smith, George P.

Type the name of the Principal Investigator/Program Director at the top of each printed page and each continuation page.

TABLE OF CONTENTS

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Smith, George P.

DETAILED BUDGET FOR FIRST 12-MONTH BUDGET PERIOD — DIRECT COSTS ONLY

FROM July 1, 1989 THROUGH June 30,

PERSONNEL (Applicant organization only) NAME	ROLE IN PROJECT	1 TYPE APPT.	2. % OF APPT.	3 EFFORT ON PROJ.	DOLLAR AMOUNT REQUESTED SALARY	FRINGE BENEFITS	
George P. Smith	Principal Investigator				22,000	5,500	27.
					12,000	3,000	15
Unnamed	Research specialist				22,000	5,500	27
Unnamed	Laboratory aide				4,800	48	4
	SUBTOTALS →				60,800	14,048	74

CONSULTANT COSTS

EQUIPMENT (Itemize) 3,

Microfuge $1,495
Microprocessor-controlled oven $1,795

SUPPLIES (Itemize by category) Isotopes $600; other specialty reagents $4000; chemicals, electrophoresis supplies $1600; small apparatus $1500; pipette tips, microfuge tubes, microtiter plates, nitrocellulose and nylon membranes $1400; petri plates, medium $1000; replacement parts $750; labware, disposable culture tubes, office supplies, other general supplies $2750 13,

TRAVEL 2 meetings 1,

PATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS

CONSORTIUM CONTRACTUAL COSTS

OTHER EXPENSES (Itemize) Contribution to service contracts for ultracentrifuge and cold rooms $2000; maintenance and repair $1000; xeroxing $400; computer services $200; phone $500; postage $150; software $500 3,

TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD (Item ... → $ 98,068

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Smith, George P.

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	1st BUDGET PERIOD (from page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL (Salary and fringe benefits) (Applicant organization only)	74,848	50,189	53,200	56,392	59,77
CONSULTANT COSTS					
EQUIPMENT	3,290	10,500			
SUPPLIES	13,600	14,416	15,281	16,198	17,1
TRAVEL DOMESTIC	1,600	1,696	1,798	1,906	2,0
TRAVEL FOREIGN					
PATIENT CARE COSTS INPATIENT					
PATIENT CARE COSTS OUTPATIENT					
ALTERATIONS AND RENOVATIONS					
CONSORTIUM/ CONTRACTUAL COSTS					
OTHER EXPENSES	4,750	5,035	5,337	5,657	5,9
TOTAL DIRECT COSTS	98,088	81,836	75,616	80,153	84,9

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD (Item 8a) ⟶ $ 420,657

JUSTIFICATION: Use [illegible] ...ces if necessary. Describe the specific functions of the personnel, consultants [illegible] all years, explain and justify [illegible] ...e items such as major equipment, foreign travel, alterations and renovations [illegible] tuition remission. For additional support requested, justify any significant increases in any category over the [illegible] period. Identify such significant [illegible] ...asses with asterisks against the appropriate amounts. If a recurring annual increase [illegible] costs is anticipated, give the [illegible] ...te. In addition, for COMPETING CONTINUATION applications, justify any significant [illegible] category over the current [illegible] ...ject.

See p. 6

Explanation of Budget

Personnel. Here is a detailed table of the personnel to be supported on this grant.

Name Title	Year 1	2	3	4	5
Smith P.I.					
Acad. Yr.	22,000				
Summer	12,000	12,720	13,483	14,292	15,150
Fringe	8,500	3,180	3,371	3,573	3,788
Unnamed Research specialist	22,000	23,320	24,719	26,202	27,774
Fringe	5,500	5,830	6,180	6,551	6,944
Unnamed Laboratory aide	4,800	5,088	5,393	5,717	6,060
Fringe	48	51	54	57	61

During the first year of the grant, I will take a sabbatical leave to work on this project; I ask for of my academic year salary and of my summer salary for that year. In years 2-5 I will devote of my academic year effort and of my summer effort to the project; I ask for none of my academic year salary and of my summer salary. The research specialist will supervise running of the lab, supervise the lab aide, train students, and perform many of the experiments. The laboratory aide will be an undergraduate, who will wash dishes, make media and simple buffers, and pour plates.

Equipment. The microprocessor-controlled forced-air oven, which can be set rapidly and accurately to any desired temperature from about 30° to 200°, will be used for long-term reactions at various temperatures, including hybridizations. The microfuge is needed to process clones and for general molecular biological manipulations; we currently have only 1, which must be carried to the cold room for samples that need to be cold (including PEG precipitated virions, a very large part of processing clones in the proposed work). In year 2 I plan to buy a replacement for a Beckman VT165 or SW50.1 rotor, which are heavily used by my laboratory.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Smith, G.P.

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Smith, George P.	Associate Professor (P.I.)	

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (Most Highest degree)	YEAR CONFERRED	FIELD OF STUDY
Haverford College, Haverford, PA	A.B.	1963	Biology
Harvard University, Cambridge, MA	Ph.D.	1970	Bacteriology/Immun
University of Wisconsin, Madison, WI	(Post-doc)		Genetics

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, exper ence, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles a complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NO EXCEED TWO PAGES.

Oct. 1963 - June 1964: Technician with Martin Nemer, Institute for Cancer Research Philadelphia.

Sept. 1964 - Feb 1970: Graduate student in Bacteriology and Immunology, under Edgar Haber, Cardiac Unit, Massachusetts General Hospital.

Feb. 1970 - July 1975: Postdoctoral Fellow under Oliver Smithies, Department of Genetics and Medical Genetics, University of Wisconsin, Madison

Sept. 1971 - Aug 1974: Helen Hay Whitney Postdoctoral Fellowship

July 1975 - Present: Assistant and then Associate Professor, Division of Biological Sciences, University of Missouri-Columbia.

Grant Support

NIH GM22686	Molecular Genetics of Antibodies	9/1/75 - 8/31/78
NIH GM25626	Molecular Genetics of Nematode Development	9/1/78 - 8/31/81
NIH AI20564	Filamentous Phage Physiology	9/1/84 - 8/31/87

SELECTED PUBLICATIONS OF G.P. SMITH

Smith, G.P. (1976) Unequal crossover and the evolution of repeated DNA sequences. Science. 191: 528-535.

Smith, G.P. (1977) The significance of hybridization kinetic experiments for theories of antibody diversity. Cold Spring Harbor Symp. 41: 863-875.

Rose, S.M., W.M. Kuehl, and G.P. Smith (1977) Cloned MPC 11 myeloma cells express two kappa genes: a gene for a complete light chain and a gene for a constant-region fragment. Cell. 12: 452-462.

Smith, G.P. (1978) Sequence of the full-length immunoglobulin kappa-chain of mouse myeloma MPC 11. Biochemical J. 171: 337-347.

Zacher, A.N., C.A. Stock, J.W. Golden, and G.P. Smith (1980) A new filamentous phage cloning vector: fd-tet. Gene. 9: 127-140.

Nelson, F.K., S.M. Friedman, and G.P. Smith (1981) Filamentous phage cloning vectors: a noninfective mutant with a nonpolar deletion in gene III. Virology. 108: 338-350.

Crissman, J.W. and G.P. Smith (1984) Gene-III protein of filamentous phages: evidence for a carboxyl-terminal domain with a role in morphogenesis. Virology. 132: 445-455.

Smith, G.P. (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the surface of the virion. Science. 228: 1315-1317.

Smith, G.P. (1987) Filamentous phages as cloning vectors. In R.L. Rodriguez and D. Denhardt (eds): Vectors: A survey of molecular cloning vectors and their uses. Stoneham, MA: Butterworth, pp. 61-85.

Michiels, F., Craig, A.G., Zehetner, G., Smith, G.P., and Lehrach, H. (1987) Molecular approaches to genome analysis: a strategy for the construction of ordered overlapping clone libraries. Computer Applications in the Biosciences 3: 203-210.

Smith, G.P. (1988) Filamentous phage assembly: morphogenetically defective mutants that do not kill the host. Virology 167, 156-165.

Bauer, M., and Smith, G.P. (1988) Filamentous phage morphogenetic signal sequence and orientation of DNA in the virion and gene-V protein complex. Virology 167, 166-175.

Parmley, S.P., and Smith, G.P. (1988) Antibody-selectable filamentous fd phage vectors: affinity purification of target genes. Gene 73, 305-318.

OTHER SUPPORT

(Use continuation pages if necessary)

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to the review of the application. If there are changes subsequent to submission, notify the executive secretary of the initial review

For each of the key personnel named on page 2, list, in three separate groups: (1) *all* currently active support; (2) *all* applications and p pending review or funding; and (3) applications and proposals planned or being prepared for submission. Include *all* Federal, nor and institutional research, training, and other grant, contract, and fellowship support at the applicant organization and elsewhere. a larger project, identify the principal investigator/program director and provide the data for both the parent project and the subp none, state "none."

For each item give: (a) the source of support, identifying number and title; (b) percentage of appointment on the project; (c) dates project period; (d) annual direct costs; (e) a brief description of the project; (f) whether the item overlaps, duplicates, or is being or supplemented by the present application; delineate and justify the nature and extent of any scientific and/or budgetary overlaps; and (g) any modifications that will be made should the present application be funded.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

(1) CURRENTLY ACTIVE SUPPORT (a)

NONE

(3) PLANNED SUBMISSIONS

RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used at the applicant organization and briefly indicate their capacities, pertinent capabilities, relative proximity of availability to the project. Use "other" to describe the facilities at any other performance sites listed in Item 9, page 1, and at sites for field st continuation pages if necessary, include an explanation of any consortium/contractual arrangements with other organizations.

☒ Laboratory: 405-406 Tucker Hall 1075 sq. ft. Equipped for standard microbiological and molecular biological research--cloning, sequencing, etc.

☐ Clinical:

☐ Animal:

☒ Computer: IBM PC with 20 Mb hard disk and math co-processor; Macintosh SE with fast communication with mainframe via cable. Software includes word-processing, graphics, HyperCard, C compiler. DNAStar DNA analysis software is available in next room. Secretarial and accounting services provided by Division of Biological Sciences and the University

☐ Office:

☐ Other (

MAJOR EQUIPMENT: List the major important equipment items already available for this project, noting the location and pe

Five ultracentrifuges with rotors; two scintillation counters; protein sequencer; numerous HPLC's; all these are in Tucker Hall. Oligonucleotide synthesizer avail at the medical school.

ADDITIONAL INFORMATION: Briefly describe any additional information describing the environment for the project. Identify support secretaries, machine shop, space, and the extent to which they will be available to the project.

The Missouri legislature has directly funded two programs in biological and medic sciences: the Program in Molecular Biology (we're in the third year) and the Food for the 21st Century Program (fourth year; this agricultural program has a strong molecular biology component). These programs benefit the research proposed here by supporting campus-wide facilities, including the oligonucleotide synthesizer.

RESEARCH PLAN

INTRODUCTION

Evaluative Material

Evaluative Material Continued

A. SPECIFIC AIMS

1. Develop techniques for constructing very large fusion-phage libraries. Two techniques will be explored. First, target DNA fragments will be ligated to linkers with non-self-complementary overhanging ends in order to increase the frequency of productive ligation products. Second, I will construct a novel composite vector in which phage λ arms flank a filamentous phage genome that can be excised in vivo with near 100% efficiency in a special "excision host." This will allow the λ packaging system to be exploited to make very large fusion-phage libraries.

2. Construct and characterize an "epitope library". This would be a 10^8-clone fusion-phage library expressing 6 codons of random coding sequence; most of the 64 million hexapeptides will be represented. The library will be tested by affinity-purifying clones recognized by anti-myohemerythrin (MHr) antibodies and sequencing their inserts.

3. *Construct and characterize "infectious antibodies". These would be fusion phage displaying a single-chain antigen-binding protein composed of the V_L and V_H variable regions of an antibody. It should be possible to affinity-purify clones whose displayed antibody binds a given antigen by reversing the usual roles of antibody and antigen.*

B. SIGNIFICANCE

1. Introduction to fusion phage.

This proposal outlines a program of development of "fusion phage," a novel expression vector developed under my previous NIH grant (Smith, 1985; Parmley and Smith, 1988; Section B). The crux of the system is that cloned antigens are displayed as part of a fusion protein on the surface of the virion itself. Antibodies can be used to affinity-purify target phage in infectious form from a 10^8-fold excess of clones displaying non-target determinants, using minute amounts of antibody; we call the affinity-purification technique "biopanning" because it exploits the binding of biotin to streptavidin. We cannot yet make libraries large enough (up to 10^{10} clones) to take full advantage of biopanning, but the prospects of 10^8-clone libraries are excellent (Section C.1). These libraries would be much larger than can be processed with conventional immunoscreening techniques, and fusion phage vectors promise to greatly simplify traditional goals like isolating clones from cDNA libraries (Parmley and Smith, 1988). In this proposal, however, I concentrate on two entirely novel technologies that seem especially suited to fusion phage.

2. An epitope library

The concept of an "epitope library" arose during a conversation about "mimotopes" with Tom McCutchan and Vidal de la Cruz (Malaria Division, NIAID). Using clever techniques for synthesizing peptides in large numbers, Geysen et al. (1986a,b) had been able to survey large numbers of short synthetic peptides for reactivity with anti-protein antibodies, and were able in this way to delineate peptides that mimick discontinuous

epitopes, which are comprised of residues distant in the polypeptide chain but adjacent in the 3-D structure. It occurred to us that fusion phage displaying short cloned peptides would be infectious analogues of chemically synthesized mimotopes, with all the advantages of replicability, clonability, and ease of surveying peptides in immense numbers; "epitope library" is my name for a mixture of phage displaying cloned peptides. Affinity-purifying clones from the library would in effect be a global search of the cloned peptides for those that bind the antibody. In contrast, the synthetic "mimotope" approach is necessarily sequential: at each of a number of steps a decision must be made to jettison all but a tiny fraction of the remaining sequence possibilities. Sequential search strategies are notorious for missing global optima in favor of local ones. The epitope library does have potential disadvantages compared to mimotopes: it could be biassed by the biology of phage production and infection, for instance (however, see Section B.3), and it does not have the freedom of incorporating D isomers and unnatural amino acids in the search for strongly binding peptides. Still, it promises to complement synthetic studies in a particularly fruitful way.

The planned 10^8-clone epitope library would display most of the 64 million possible hexapeptides (Section C.2.c). Since epitopes typically include about 6 residues and tolerate many replacements, the hexapeptide library might contain epitopes recognized by most anti-protein antibodies. The library will be characterized with polyclonal and monoclonal antibodies directed against myohemerythrin (MHr), a 118-residue protein whose three-dimensional structure and antigenic makeup have been intensively studied (Section C.2.d). The antibodies will be used to affinity-purify cognate clones from the library, and the amino-acid sequences of the inserts in those clones will be determined at the DNA level. The sequence data will be used to address three questions of theoretical and technological interest: (a) Do all or most antibody-reactive inserts have recognizable similarity to features of Mhr 3-D structure, including potential discontinuous epitopes (Section C.2.e)? Never before has a search for cross-reacting, structurally unrelated epitopes, or for discontinuous epitopes, been conducted on a scale that comes within orders of magnitude of this--even if the biology of phage "censors" many more potential epitopes than I consider remotely plausible. If a substantial fraction of the antibody-reactive peptides do resemble MHr, this system might be used to map epitopes--including discontinuous ones--on other proteins of known 3-D structure, without the need to clone segments of the gene or synthesize peptides. (b) Do the clones purified with polyclonal anti-MHr antibodies have enough information by themselves to single out the MHr protein from a large database of protein sequences (Section C.2.f)? If so, it may be much easier in some situations to identify the protein recognized by an antibody with the epitope library than by actually cloning the gene or its mRNA. Obviously it only applies to species for which extensive sequence information is available--e.g., humans in a few years, and, by homology, mammals in general. (c) Do clones purified with monoclonal anti-MHr antibodies have enough information to identify epitope(s) responsible for binding (Section C.2.g)? If so, it may be possible to use an interesting antibody (one, say, that protects against a disease) to purify cognate clones from the epitope library, and from the

sequences of the inserts deduce the epitope recognized by the antibody--all without having to clone the gene encoding the target protein or obtain any information about the protein's structure. Such information could be used, for instance, in the design of peptide immunogens; indeed, the fusion phage itself can serve as immunogen for some purposes (de la Cruz et al., 1988).

3. *A library of "infectious antibodies" (Section C.3).*

This would be a library of fusion phage displaying, not foreign antigens, but rather antibodies with a great diversity of antigen-binding specificities. The cloned antibodies would be the 225-residue single-chain antigen-binding proteins ("single-chain antibodies," or scAb's, as I will call them) recently reported by Bird et al. (1988). Phage-borne antibodies hold out the promise of completely bypassing animals and mammalian cell culture in the generation of monoclonal antibodies with defined specificities. Instead, the library of antibodies would be generated in vitro, using degenerate synthetic oligonucleotides to encode the six short complementarity-determining regions (CDR's) that determine the specificity of antibodies; hopefully the library as a whole would represent a great diversity of different binding specificities. From this library, phage displaying any desired binding specificity would be affinity-purified by reversing the role of antigen and antibody in biopanning. The technological advantage of this way of producing antibodies is obvious: it would be incomparably cheaper and easier, would avoid the sacrifice of animals, and would circumvent the censorship that the intricacies of immunogenicity exert on the repertoire of possible antibodies.

C. PROGRESS REPORT

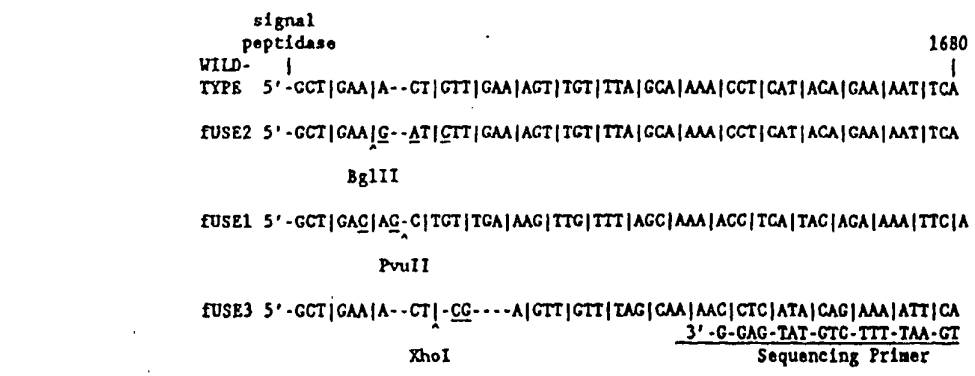
1. Introduction: fd-tet

The filamentous virion (reviewed recently by Webster and Lopez, 1985; Model and Russel, 1988; and myself, 1987) consists of a stretched-out loop of single-stranded DNA (ssDNA) sheathed in a tube composed of the major coat protein. Four minor proteins are found at the tips of the virion, pIII (product of phage gene III) being of particular interest here since it is the capsid protein that bears foreign amino acids in fusion phage. The phage infects cells displaying an F pilus, the entering ssDNA loop being converted to a double-stranded replicative form (RF), which replicates and eventually serves as template for progeny ssDNA. Somehow, by a process that is still mysterious, the ssDNA is extruded through the inner membrane, concomitantly acquiring the virally coded coat proteins, some (probably all) of which are membrane proteins. This process is not lethal to the host: infected cells continue to multiply, albeit at a slower rate (hence plaque-formation).

Phage fd-tet, originally constructed as a cloning vector in my laboratory (Zacher et al., 1980), confers tetracycline resistance on the host and thus can be propagated like a plasmid independently of phage function. I discovered that mutants that are completely defective for assembly nevertheless can be propagated (Smith, 1988), whereas such mutants in other strains of filamentous phage kill the host (Pratt et al., 1966; Hohn et al., 1974). The absense of cell killing facilitates the study of assembly by making it easy to construct a variety of defective mutants and study them without the confounding factor of cell morbidity (Grissman and Smith, 1984; Smith, 1988; Bauer and Smith, 1988). More importantly in the present context, it has made possible construction of the fUSE vectors described in the next section.

2. Design of fUSE vectors.

Under my previous grant I showed that pIII tolerates foreign amino-acid inserts, producing so-called "fusion phage" that retain phage function and display the foreign amino acids on the surface (Smith, 1985). I envisioned two major uses for fusion phage: as novel immunogens for eliciting immunity to the foreign amino-acid segment; and as "antibody-selectable" expression vectors, enabling rare clones bearing a target antigenic determinant to be affinity-purified from a large library of clones bearing many different determinants. Fusion phage have been explored as immunogens by de la Cruz et al. (1988), using determinants from malaria parasites. My group concentrated on devising an antibody-selectable vector system; the result is the fUSE vectors (Parmley and Smith, 1988), which are derived from fd-tet (Section 1) and carry their gene-III insert just downstream of the signal peptide. Here is the relevant region of gene III for fd-tet (with the wild-type sequence) and three vectors, fUSE1, fUSE2 and fUSE3:



The gene-III reading frame is disrupted in fUSE1 and fUSE3, abolishing all pIII functions, including infectivity; the vectors are nevertheless propagatable as plasmids because of the absense of cell killing in fd-tet (Section 1). Some of inserts spliced into these vectors (usually a minority) restore the gene-III reading frame and thus infectivity; these frame-restoring inserts, as well as the clones harboring them, will be called productive (fUSE1 and fUSE3 themselves are nonproductive). If the reading frame in a productive insert coincides with a natural reading frame, the resulting hybrid gene-III protein will contain a foreign amino acid sequence that is part of a natural protein; such inserts and the corresponding clones will be called meaningful. Vector fUSE2 has a single BglII cloning site that does not disrupt the gene-III reading frame. Some of the fragments cloned into that site (again, a minority in most cases) will preserve the gene-III reading frame. As in the case of fUSE1 and fUSE3, inserts and clones can be either productive or nonproductive, and the productive inserts and clones can be either meaningful or meaningless.

Infective phage are detected as tetracycline transducing units (TU): that is, cells are infected with the phage and, after a gene-expression period, spread on plates with tetracycline. This minimizes the demand on infectivity (which requires pIII), since a single successful infection yields a visible colony. Nonproductive phage are noninfective and therefore give no transductants; they make no contribution to the library, since clones are ultimately detected as TU. This eliminates the large background of nonproductive clones, including clones without inserts in frame-shifted vectors like fUSE1 and fUSE3.

3. Performance.

The following table summarizes the performance of fUSE vectors with inserts of varying size, all of which have been sequenced with the primer shown in the diagram above (Parmley and Smith, 1988; Smith, unpublished):

Strain	Insert size (bp)	Yield (particles/ml)	Infectivity (tu/particle)
fd-tet	---	7×10^{11}	ND
fUSE1	0	$\geq 1 \times 10^{11}$	0
fUSE2	0	1×10^{12}	0.27
fUSE1-T7	20	4×10^{11}	0.30
fUSE2-NANP	54	5×10^{11}	0.13
fUSE2-BACK	54	5×10^{11}	0.06
fUSE2-lac54	*54*	*ND*	*ND*
fUSE1-lac71	71	1×10^{12}	0.13
fUSE2-lac78	*78*	*ND*	*ND*
fUSE1-lac335	335	5×10^{11}	0.012

It is apparent that inserts have almost no effect on phage yield, which is about 25% that of the popular M13mp vectors. Four of the clones studied for infectivity, including two that express unnatural peptides (fUSE1-T7 and fUSE2-BACK), are essentially wild-type in this respect, and the remaining clone, fUSE1-lac335, is only about 20-fold less infective (see Section C.3.d for a discussion of this clone). It is noteworthy that fUSE1-lac335 has the longest insert (112 amino acids), but it is too soon to tell whether reduced infectivity is mostly a function of insert size.

Clones *fUSE2-lac54*, fUSE1-lac71, *fUSE2-lac78* and fUSE1-lac335 express segments of the β-galactosidase protein, and all four react with anti-β-galactosidase antibody (Section 4); clone fUSE2-NANP displays the repeated epitope of the Plasmodium falciparum circumsporozoite (CS) protein, and reacts with anti-CS antibody (V. de la Cruz, unpublished); and two EcoRI endonuclease peptides cloned into other vectors react with anti-endonuclease antibody (Smith, 1985; S. Parmley, unpublished). So far, then, seven fusion-phage clones have been tested with potentially reactive antibodies, and all seven have reacted.

These results support three encouraging conclusions about the performance of fUSE vectors. First, many completely different inserts--all we have tried, in fact--are compatible with wild-type yield. This is not to say that some inserts won't interfere with incorporation of the hybrid pIII into the virion; in particular, a recombinant pIII with a spurious "stop transfer" sequence (an extended stretch of hydrophobic residues that anchors the polypeptide in the membrane) will not be properly inserted into the inner membrane, where morphogenesis occurs (Davis and Model, 1985). Evidently, however, such inserts are very much the exception, not the rule. Likewise (and second), many completely different inserts are compatible with high infectivity, though the reduced infectivity of fUSE1-lac335 noted above indicates that some clones may be somewhat underrepresented on this score. While we must anticipate the possibility that differential infectivity will introduce some quantitative bias in a fusion-phage library, it is most unlikely in light of our results that a substantial fraction of clones from a fusion-phage library will be fully censored--even clones expressing unnatural peptides (like the proposed epitope library discussed in Section C.2). Third, most phage display their cloned peptide on the virion surface, as do all seven clones we tested. In summary, only

a small minority of inserts will be censored by the biology of phage production and infection, or inaccessible to antibodies.

4. Biopanning: an effective method for affinity-purifying target clones.

Phage in a primary library are reacted with microgram amounts of biotinylated antibody, and "panned" for 10 min with a polystyrene petri plate that has been coated with streptavidin and blocked with excess BSA. Virions to which antibody binds are in turn attached to the plate via the super-strong biotin-streptavidin reaction. After unbound phage are washed away, bound phage are eluted with acid and neutralized with base. In model experiments with biotinylated anti-β-galactosidase, this simple procedure results in approximately 10^5-fold enrichments of both fUSE1-lac335 and fUSE1-lac71 over wild-type phage, with 2-4% yield. Identical results were obtained with biotinylated antibody affinity-purified from a Western blot (Smith and Fisher, 1984); thus monospecific antibodies can be readily isolated from polyspecific antisera in sufficient quantity to biopan a large library. We have also used a biotinylated second antibody, obviating the need to biotinylate each individual primary antibody.

When needed, 2-3 three rounds of biopanning, with an intermediate amplification as a plate stock, can yield even higher enrichments. In one experiment a model library containing only 1000 TU of fUSE1-lac335 and 10^{11} TU of fUSE2 was enriched 13 million fold for fUSE1-lac335; the fUSE1-lac335 clones comprised 12.5% of the total. This proves it is easy to affinity-purify clones that are about 1000 times rarer than those isolated in conventional expression vectors like λgt11 (Young and Davis, 1983).

Clones fUSE2-lac54 and fUSE2-lac78 were obtained by biopanning a 12,000-clone library of random Sau3A fragments from total E. coli chromosomal DNA, using blot affinity-purified anti-β-galactosidase. Three clones were sequenced, two corresponding to fUSE2-lac54 and one to fUSE2-lac78; no inserts from elsewhere in the E. coli genome were obtained, even though meaningful inserts from the lacZ gene theoretically comprised only about 1/6000 of all possible inserts.

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Smith, G.P., Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 228, 1315-1317, 1985.

Smith, G.P., Filamentous phage as cloning vectors. In Vectors: A Survey of Molecular Cloning Vectors and their Uses, R. Rodriguez and D. Denhardt, eds., Butterworth Press, 1987, pp. 61-85.

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D. EXPERIMENTAL DESIGN AND METHODS

1. Two strategies for constructing 10^8-clone libraries

a. Linker tailing/partial fill-in. The goal here is to put non-palindromic single-stranded ends on the vector, while the target fragments are tailed with another set of non-palindromic ends complementary to the ends on the vector; this increases the yield of productive ligation products by preventing target-target and vector-vector splicing. An example will serve to illustrate the strategy. Vector fUSE3 has a single XhoI cloning site that also introduces a frameshift into gene III (Section B.2). After cleavage with XhoI, the 4-base, 5' overhang is partially filled in with Klenow fragment and dTTP to generate a 5'-TCG end. Meanwhile, blunt-ended target fragments are ligated with a vast molar excess of hybridized linkers 5'-CGAGGCGCCGCCGGC-3' and 5'-GCCGGCGGCGCC-3'; when the former is base-paired with the latter it has a 5'-CGA overhang, which is complementary to the 5'-TCG ends on the vector. The linkers are spliced quantitatively to the targets at their blunt ends under the conditions of Lathe et al. (1984), and the linker-tailed target fragments size-fractionated and ligated to the partially filled-in vector; only vector-target ligations are permitted, increasing the yield of productive ligation products. A similar design was recently reported by Aruffo and Seed (1987), increasing the probability of success. For reasons that have to do with the planned epitope library (Section 2.c) the linkers are designed so that a target will restore the gene-III reading frame iff it has an integral number of codons. The foreign amino acids will be preceeded by Arg-Gly-Ala-Ala-Gly and followed by Ala-Gly-Gly-Ala-Ser; the (Ala,Gly) tetrapeptides are designed to be essentially structureless, insulating the insert from possible "carrier effects" exerted by pIII.

b. λfUSE vectors. In vitro packaging of recombinant λ DNA into phage particles is much more effective for making large libraries than transformation with plasmid or filamentous phage DNA; Smithies et al. (1985), for instance, packaged 1.4-6.3 x 10^8 primary clones with conventional techniques. In order to exploit λ packaging, I will construct a 45.6-Kbp composite λfUSE vector, consisting of a fUSE genome flanked by the left arm of λEMBL3 and the right arm of λcI857Sam7 (Sam7 prevents lysis in non-amber-suppressing hosts). The fUSE moiety will supply a Chi site, will have a unique gene-III XhoI site like fUSE3 (Section B.2), and will be excisable in vivo as described in the next paragraph.

Excision will be effected by Cre, a phage P1-encoded site-specific recombinase with high specificity for the 34-basepair loxP site (Sternberg and Hamilton, 1981; Sternberg et al., 1986). Accordingly, the fUSE moiety of λfUSE will be flanked by loxP sites, and the host will be lysogenic for a restrictionless strain of phage P1. When this "excision host" is infected with λfUSE particles, Cre-catalyzed recombination excises the fUSE genome, which replicates in the filamentous phage mode, in effect "converting" a λ clone to a filamentous phage clone. The reciprocal product of recombination, though too small to package (36.6 Kbp), will encode λ lytic functions that might kill the cell; the excision host is therefore lysogenic for λ as well as P1. The excision host will be

defective for EcoK restriction, and also F⁻ and thus uninfectable by filamentous phage; the latter trait prevents propagation of filamentous phage via infection and should reduce selection pressure against slight defects in pIII, which is required for infectivity. For reasons that have to do with the epitope library (Section 2.c) the excision host will be supF. The λfUSE vector itself can be propagated in the λ mode on a supF strain (to suppress Sam7) that is nonlysogenic for P1 or λ. David Hoad (Promega Biotec; personal communication) tried the Cre-loxP system with a model composite vector in which the excisable moiety was a plasmid rather than a filamentous phage. A substantial fraction (30) of the cells infected with the phage formed antibiotic-resistant (i.e., plasmid-harboring) colonies, despite two defects of the excision host: the P1 prophage was not restrictionless, and there was no λ prophage to repress λ lytic functions. I therefore assume at least 30 efficiency, and an efficiency closer to 1000 seems more likely.

To use λfUSE, cleave at the XhoI cloning site, partially fill in with Klenow and dTTP (this should not affect the two λ sticky ends, since the first nucleotides to be filled in are A and G), ligate with linker-tailed target fragments (previous section), and package the resulting concatamers in vitro into λ particles. If necessary, the λ particles can be amplified in the λ mode to compensate for a low efficiency of excision (next paragraph). The λ particles are then infected into a log-phase culture of the excision host, and after a gene-expression period tetracycline is added and the culture allowed to grow to stationary phase. The filamentous virions secreted into the medium constitute the fusion-phage library; assuming a typical titer of 5 X 10^{10} TU/ml, a 10-liter culture should yield a library in which 10^8 clones are represented by an average of 5 X 10^6 TU each--enough for 5000 biopannings. The number of primary productive clones actually represented in the fusion-phage library will be monitored by plating an aliquot of the culture just after tetracycline addition, and ascertaining the fraction of colonies that secrete infectious virions. There is always the danger when preparing stocks in liquid culture that fast-growing clones will overtake the culture. For small libraries this can be overcome by amplifying phage as plate stocks, but that would be a major inconvenience for 10^8-clone libraries. The two countermeasures incorporated in my plan--infecting the excision host in log phase, when cells are physiologically relatively uniform, and using F⁻ cells to minimize selection on the basis of pIII function--should prevent gross disparities. In any case, the hexapeptide inserts in the epitope library should have minimal effect on growth of cells, which does not require gene III function (Section C.1), or on phage yield (Section B.3).

The primary library of λ particles could if necessary be amplified in the λ mode before being "converted" into filamentous phage by the excision host. The following two-host scheme would ensure that every clone goes through exactly two cycles of infection in liquid culture, with no possibility of competition between clones. Host 1 would be supF, which suppresses Sam7 and permits release of phage, and also streptomycin sensitive; host 2 would be non-amber-suppressing, leading to intracellular accumulation of progeny phage, and also streptomycin resistant. The primary, in-vitro-packaged library (10^8 λ particles) would first be

infected into 10^9 log-phase host 1, and after sufficient period for phage absorption the culture would be diluted into 10 liters containing 10^{12} host 2 in log phase. Of the phage released by host 1, 99.9% will infect host 2, since it's in 1000-fold excess; the few phage that do infect the remaining host 1 cells will be killed by adding streptomycin after the first round but before the second round of infection is complete--the timing will not be critical. After sufficient time for the second round to finish (there is no harm in allowing too much time) cells are harvested and lysed artificially to give the amplified λ library. Pre-amplification is attractive for another reason, even if not required to compensate for low excision efficiency: the tiny gene-III inserts should have even less effect on growth in the λ mode than on growth in the filamentous phage mode.

2. An epitope library

A polyclonal anti-protein antibody is a complex mixture of species. The mixture as a whole has high specificity for the antigen, but this degree of specificity might not extend to the individual component species. The binding requirements for those epitopes that have been investigated in detail are not high, typically only 3 amino acids being critical. Such loose requirements, one would think, will be met by many other proteins, and that squares with frequent anectodes about monoclonals that cross-react with the "wrong" proteins. It is also entirely possible, but as yet unproven, that most individual antibodies can bind multiple epitopes with no structural similarity to one another. Polyclonal antibodies are highly specific despite these potential cross-reactions because their component species recognize many different epitopes, and even recognize the same epitope in different ways; thus the cross-reactions of the various antibody species with unrelated antigens are largely uncorrelated, so that each is quantitatively minor. Talmage (1959), in a seminal paper, pointed out that this "combinatorial specificity" (as I later called it in my book on antibodies; Smith, 1973) could explain how a specific response might be mounted against an unlimited universe of antigens, with only a limited repertoire of individual binding-specificities.

If the repertoire of specificities is indeed limited, it might be partially or fully encompassed in random hexapeptides displayed in a 10^8-clone epitope library. By biopanning the library with an antibody and sequencing the inserts in the resulting clones, we sample the epitopes recognized by the antibody's component species. This information, even if biassed (and for the reasons spelled out in Section B.3 I don't think it will be), promises a far broader, more global investigation of antibody specificity than has been possible hitherto, and could well have technological applications.

a. Will we succeed in affinity-purifying clones from the epitope library? Our success with *fUSE2-lac54*, fUSE1-lac71, and *fUSE2-lac78*, which display only *19*, *24* and *27* residues of β-galactosidase, respectively, already shows that short, presumably flexible peptides serve as effective targets for biopanning. In light of hundreds of published studies of the binding of antibody to very short peptides (reviewed by Getzoff et al., 1988), assayed in the usual way by ELISA, there is every reason to

extrapolate this result to the hexapeptides displayed by the epitope library. The binding requirements for successful biopanning are essentially those for a positive ELISA signal: reasonable forward reaction rate, coupled with stability to multiple washing steps. I assume antibodies will bind the phage-borne peptides as they do peptides conjugated to any other carrier via a structureless linker. Since the maximum concentration of antibody in biopanning is about 10 μM, peptides with dissociation constants smaller than about 100 μM should be purifiable in some yield; by reducing the antibody concentration, peptides with higher affinities can be preferentially purified. Dissociation constants actually observed with peptide-reactive antibodies typically lie in the micromolar range or less. Fieser et al. (1987), for instance, measured dissociation constants in the range 0.05-4 μM for anti-peptide antibodies reacted with whole MHr, and from their binding competition data I calculate constants in the range 0.01-3 μM for the same antibodies reacted with the free peptides.

Most studies of the binding of antibodies to peptides have focussed on short sequential peptides (typically hexamers) representing successive residues in the parent antigen's primary sequence (Getzoff et al., 1988). Most polyclonal anti-protein antibodies, and all the polyclonal antibodies I'll use, bind well to at least some sequential peptides. Some monoclonals, including three that will be used in my study (next section), are known to bind sequential peptides, too. Typically several residues in a sequential hexapeptide can be replaced without affecting binding. For this reason I have little doubt I will succeed in obtaining some affinity-purified clones to analyze, despite the fact that some hexamers will be absent from the library. Even if sequential peptides are the only antibody-binding peptides, the results will still be of considerable immunological interest (Section e) and potential applicability (Section f).

Less clear, however, is whether most monoclonal antibodies will bind clones in the library. Apparently only a minority of monoclonal antibodies, and a minority of the individual species in a polyclonal antibody, bind sequential peptides (reviewed in Benjamin et al., 1984). An obvious explanation is that the antibodies that fail to bind sequential peptides recognize discontinuous epitopes. Whether or not that's so, the success (so far) of Geysen's "mimotope" technique (Section A.2) encourages the hope that the epitopes recognized by such antibodies can be effectively mimicked by peptides that don't represent successive residues in the parent protein. If so, we might expect most individual molecular species of anti-protein antibody to bind some clones in the epitope library. That would widen the applicability of the epitope library (Sections e and g).

b. A miniature hexapeptide library. I am currently constructing a miniature epitope library with only the 4096 hexapeptides encoded by the last 18 positions of the oligonucleotide 5'-CGAGNTTNTCNCGNGANAANC-3', where N stands for an equimolar mixture of the 4 nucleotides. The 12 nondegenerate residues in these 18 positions are palindromic, so when the oligonucleotide is self-hybridized it forms an 18-basepair duplex flanked by 5'-CGA overhangs; this permits it to be cloned into fUSE3 (Section 1.a). Only a small library is required, so the experiment need not await development of λfUSE vector. One of the peptides in the library, DFLEKI,

corresponds to positions 79-84 of myohemerythrin and is known to bind 3 monoclonal and 5 polyclonal antibodies (Geysen et al., 1987; Fieser et al., 1987). The library will be biopanned with these antibodies to see which among the 4096 sequences they bind. If, contrary to expectation, biopanning is not successful, the insert will be flanked by structureless linkers, as planned for the main library (Section c); this might alleviate "carrier effects" (if any) exerted by the nearby pIII domain (Section 1.a).

c. The main hexapeptide library. Random hexapeptides will be encoded by dimers of the degenerate olignucleotide 5'-(NJN)$_3$-3', where N is as in the previous section and positons J have composition 30% A, 30% T, 20% C and 20% G. These compositions were chosen by computer to (nearly) maximize the uniformity of representation of amino acids codod by the 9-mer in a strain carrying the supF (tyrosine-inserting) amber suppressor, subject to the constraint that the 9-mer have a "palindromic composition" (% A at position 1 of the trimer = % T at position 3, and % A = % T at position 2), so that each individual 9-mer sequence and its complement are equally represented. Phosphorylated, concentrated 9-mer will be self-hybridized under stringent conditions (cooling over several days) to ensure that the rarest individual sequences (1/512,000) will hybridize fully. The hybridized preparation will be mixed with the hybridized linker described in Section 1.a and polymerized at 4° with ligase. Since the 5'-CGA ends of the linker cannot self-ligate, the limit product will be a series of molecules flanked at both ends by linker, and having 0, 1, 2,... tandem 9-mers between. The multimers will be fractionated by acrylamide gel electrophoresis, and the (presumably discrete) bands excized and eluted. The 6-codon fragments will be spliced into the XhoI site of λfUSE as in Section 1.a, and packaged and infected into a supF excision host as in Section 1.b to generate a hexapeptide library. All inserts should ligate in the correct reading frame at both ends, and because of the "palindromic composition" of the 9-mer the orientation doesn't matter. In principle 92% of the clones should be productive and meaningful--the fraction of inserts without TAA or TGA stop codons not suppressed by supF. Some of the 64 million hexapeptides will be missing in a 10^8-clone library, especially as the 20 amino acids will not be exactly equally represented. Still, enough different hexapeptides should be present to mimick almost any epitope (Section a). (It may be necessary to make libraries with larger peptides in order to repesent a greater number of epitopes.)

Since all clones carry small inserts of exactly the same size, and since very few hexapeptides should chance to have a physiological effect on the cell, nearly all clones should have yields and infectivities that are close to one another and to the yield and infectivity of fd-tet without an insert. For this library, therefore, phage biology should impose little bias (Section B.3), and a liquid stock should give adquately uniform representation of the clones (Section 1.b).

d. Isolating and sequencing clones reactive with anti-MHr antibody. Myohemerythrin (MHr) is a 118-residue monomeric protein whose 3-D structure is known, and whose antigenic structure has been intensively studied with synthetic peptides (Tainer et al., 1984, 1985; Geysen et al., 1987; Getzoff et al., 1987; Fieser et al., 1987). I plan to charactorize the epitope

library using polyclonal and monoclonal antibodies against MHr. Most of the chosen antibodies bind sequential peptides, but I will also include monoclonals that don't. Polyclonal antibodies will be affinity-purified prior to use in biopanning, using as immunosorbents both native protein bound to a column and denatured protein in a Western blot band; antibodies from the blot should be enriched for species recognizing sequential peptides. Polyclonal antibodies will be blocked with excess UV-killed, non-tetracycline-resistant phage carrying a linker dimer with no insert, to neutralize antibodies that react with determinants other than the cloned peptide (e.g., one of the approximately 400 tripeptides on the phage coat proteins). All antibodies will be used to affinity-purify phage from the main hexapeptide library. Representative affinity-purified phage will be propagated and the amino acid sequences of their inserts analyzed from three points of view, as detailed in Sections e-g below.

e. New evidence on the nature of antibody specificity. I anticipate two categories of epitope among the antibody-selected clones. First, there will be epitopes, both continuous and discontinuous, with recognizable similarity to features of MHr--particularly its surface. Finding these will be greatly aided by consultation with Hannah Alexander, who now lives in Columbia, and who did much of the key work with MHr under Richard Lerner; and with Richard Lerner himself, who has also agreed to supply the requisite antibodies. (In the appendix I include letters of support from both these investigators.) If this search is successful, it would suggest that the epitope library can be used to map epitopes on proteins of known 3-D structure without the expense and labor of synthesizing and testing individual peptides (or subcloning segments of the gene). Second, as explained in the introduction to Section 2, there may well be epitopes with no recognizable similarity to MHr; these may or may not fall into recognizable families of similar sequences. Whatever the fraction of the two types of epitope, we will have learned something important about antibody specificity--something that would have been difficult to learn in other ways. For both types of epitope, it will be valuable to see if the allowable sequence variability of peptides from the library is consistent with that found with synthetic peptide studies--particularly "replacement nets," in which each residue in a peptide is systematically replaced with the other 19 amino acids (Getzoff et al., 1987). Lastly, we can ask if the three monoclonals that bind the DFLEKI epitope (Section b) bind the same clones in the library; "micropanning," a miniaturized version of biopanning (Parmley and Smith, 1988), will allow all clones isolated with one of the antibodies to be rapidly tested with the other two.

f. Do sequences reactive with polyclonal antibodies contain enough information themselves to single out MHr from a large protein-sequence data-base, without using the information that MHr was the eliciting protein? Cloned peptides that have been affinity-purified with a polyclonal antibody are images of sequence elements of the protein recognized by the antibody. Even though the image is vague and unreliable, the information from all clones combined may well be enough to single out the correct protein sequence, provided it exists somewhere in a database. This would be especially so if the antibody had been purified from a blot and was thus enriched for species recognizing continuous epitopes. I will

develop search algorithms based on observed relationships between inserts and MHr sequences; these will then be tested with clones biopanned with antibodies against other proteins of known amino acid sequence.

I think this endeavor is technologically interesting for two reasons. First, we'll soon know the sequence of most human proteins, and thus (by homology, with a few replacements) most mammalian proteins too. Second, antibodies with defined specificities may in the future be easy to isolate as a result of the development of genes for single-chain antibodies (scAb's) expressed in E. coli (Bird et al., 1988; Section 3).

g. Do the sequences in clones reactive with a monoclonal antibody contain enough information themselves to identify the epitope, without using knowledge of MHr structure? The ability to identify the epitope recognized by a monoclonal (or monospecific) antibody means immunogens capable eliciting antibodies of similar specificity (perhaps the fusion phage itself) might be obtained without having to isolate usable quantities of the gene, its transcript, or its protein product. This approach might be particularly useful if the epitope is discontinuous, since such determinants are difficult to identify even when the primary sequence of the polypeptide is known; or if mRNA encoding the target protein is difficult to obtain (e.g., malaria sporozoite proteins).

h. Comparison of fUSE and λgt11 as vectors for the epitope library. In at least two respects, λgt11 (Young and Davis, 1983) would be greatly inferior to fusion phage for this purpose. First, we would have to be content with many fewer clones. Screening a 10^6-clone λgt11 library would be impractical, and would consume huge amounts of antibody; at the plaque density recently recommended by Snyder et al. (1987), for instance, the initial screening would require 1000 150-mm petri plates! By contrast, we probed a mock 10^8-clone fusion-phage library with three biopannings and an intermediate amplification in a few hours over a four-day period, with no more antibody than could be eluted from a Western-blot band (Parmley and Smith, 1988; Section B.4). A second, possibly very significant, advantage of biopanning over screening is that background reactions of antibody with non-phage impurities can't interfere--only reactions with phage are scored.

3. *A library of "infectious antibodies"*

a. Cloning an anti-fluorescein scAb into fusion phage. Single-chain antibodies (scAb's) are antigen-binding proteins expressed in E. coli; the polypeptide comprises the V_L and V_H variable regions of an antibody, the C-terminus of V_L being linked via a designed peptide to the N-terminus of V_H (Bird et al., 1988). In collaboration with Bird, I plan to clone a fluorescein-binding scAb into fusion phage, the scAb moiety being linked to pIII via a trypsin-sensitive peptide for reasons that will become apparent in the next section. This scAb phage--an example of the "infectious antibodies" envisioned in Section A.3--will be used as the model to optimize the performance of scAb phage in general. Because the overall conformation and physical characteristics of antibodies are determined largely by the framework residues, I assume that conditions for optimizing performance of one scAb phage also apply to others, expressing different CDR's and therefore different binding specificities.

The scAb phage will be assayed initially for infectivity and ability to be affinity-purified using biotinylated fluorescein in place of biotinylated antibody in the standard biopanning protocol. It may also be possible to detect fluorescein binding by fluorescence quenching; if so, that would be a much easier way of processing multiple samples. I will attempt to assess non-specific "stickiness" by biopanning with a biotinylated mixture of proteins--e.g., total E. coli proteins. In Sections b-e I will discuss responses to various problems I may encounter.

b. Can infectivity be enhanced by cleaving off the scAb moiety? If the infectivity of scAb phage proves to be low or nonexistent, the problem could well be that the bulky scAb moiety interferes with the function of pIII during infection. I would try to restore infectivity by cleaving off the scAb moiety with trypsin (the infectivity of wild-type phage is not affected by trypsin; Salivar et al., 1964). It would be easy to implement this remedy if it works: affinity-purified phage would simply be digested with trypsin before being infected into the final host.

c. Renaturation. When expressed at high levels in E. coli, scAb's must renatured before acquiring antigen-binding activity (Bird et al., 1988). This treatment may be unnecessary for scAb phage, since in that case the scAb protein is made in minute quantities and secreted directly into the periplasm--a process with a prima facie resemblance to secretion of natural antibodies into the endoplasmic reticulum. If, nevertheless, renaturation turns out to be necessary, the prospects for success are enhanced by the resistance of filamentous phage to sulfhydryl reagents and urea concentrations as high as 6 M (unpublished observations).

d. Proteolysis of pIII fusion proteins. Western-blot analysis of recombinant pIII from fUSE1-lac335 virions showed extensive proteolysis. Since pIII functions in infection, the observed degradation might explain the 20-fold reduced infectivity of fUSE1-lac335 virions (Section B.3). Before incorporation into the virion, pIII is a membrane protein with all but a short C-terminal tail in the periplasm. The proteolysis noted in fUSE1-lac335 must therefore be either periplasmic or extracellular, not cytoplasmic (E. coli degrades a wide range of abnormal periplasmic proteins; Strauch and Beckwith, 1988). Although neither proteolysis nor reduced infectivity precludes effective affinity purification of clone fUSE1-lac335, they might present severe problems in the case of scAb phage, whose inserts are twice as long as the β-galactosidase insert in fUSE1-lac335. On the other hand, if the scAb moiety spontaneously folds into its compact native conformation, it might be particularly resistant to proteolysis, as are natural antibodies. Indeed, natural proteolysis might have the salutary effect of removing improperly folded scAb moieties.

Because of its possible beneficial effects, I hope to avoid interfering with natural proteolysis. If all else fails, however, and if Western blot analysis indicates extensive degradation of the recombinant pIII, I will introduce into the host the degP4::Tn5 mutation recently described by Strauch and Beckwith (1988). That mutation stabilizes a wide variety of abnormal periplasmic proteins that are rapidly degraded in

wild-type cells. Alternatively or additionally, I will include protease inhibitors in the medium during propagation--e.g., p-aminobenzamidine inhibits several membrane-bound proteases (Palmer and St. John, 1987).

e. Enhancing performance with phage and host mutations. Low infectivity or inability to be affinity-purified might be overcome by phage mutations--for example, mutations in the scAb moiety that stabilize the protein without interfering with antigen binding. Accordingly, λfUSE particles with the scAb insert will be treated with mutagens and infected into the excision host, the resulting fusion phage being biopanned with biotinylated fluorescein. The affinity-purified clones, if obtained, should be highly enriched for mutants with enhanced overall performance. If this enrichment yields no such clones, I could select instead for infectivity by infecting a limiting number of unfractionated phage directly into cells.

Host mutations (other than deg4::Tn5 mentioned above) might also enhance performance of scAb phage, by blocking proteolysis or other means. If it seems worthwhile, I will mutagenize a culture of excision host cells, infect them with scAb-bearing λfUSE particles, and screen 5-10 thousand tetracycline-resistant clones directly on plates for the ability to secrete infectious phage. The screen exploits the fact that colonies harboring fd-tet, which confers resistance to tetracycline, are surrounded by small satellite colonies when plated on tetracycline medium along with a large number of tetracycline-sensistive, infectable cells (secondary host). The satellites arise from infection of the secondary host with phage secreted on the plate by the primary colony. If the infectivity of scAb-bearing phage is severely reduced, no such satellites will appear; while mutations that restore infectivity should restore satellites. Satellites should be detectable on crowded plates as blue colonies by using a lacZ⁻ primary host, a lacZ⁺ secondary host, and Xgal and IPTG in the medium.

f. A critical test. I will clone a number of other scAb's of known antigen-binding specificity. These phage will be mixed to make mock libraries in which one antigen-binding clone is present in minute numbers, the rest being present in large and equal titers. Biopanning such a library with biotinylated antigen specific for the rare clone would in all essential respects resemble biopanning a real library of scAb phage. Although in the mock library only a few clones would contribute to the background, non-specific binding presumably will not depend critically on the exact structure of the CDR's, and thus should be adequately represented by a modest number of clones.

g. On constructing the library. This is probably 5 years in the future, so only a bare outline will be presented. I envision the scAb gene being spliced together from 11 synthetic oligonucleotides encompassing the 6 CDR's and the 5 framework regions that lie between the first CDR of the L chain and the last CDR of the H chain. All components would have long, unique overhanging ends so they could be ligated in only one way. The oligonucleotides encoding the CDR's would be degenerate and (for most CDR's) heterogenous in length in order to incorporate diversity in binding specificity. After ligation the construct would be gel purified, ligated

into a λfUSE vector that already has the missing N- and C-terminal framework residues, and packaged into λ particles to make the fusion-phage library as outlined in Section C.1.b. The diversity in the library might be further increased by treating the fusion phage with mutagens and/or passaging them many times through infectable cells to promote recombination. After renaturation of the displayed scAb's, if necessary, the library would probably have to be treated somehow to remove aggregated and generally "sticky" phage; one possibility would be to pass the phage through nitrocellulose filters to which a mixture of proteins or other antigens has been fixed (filamentous phage pass through 0.45 μm filters). The library would be biopanned with a wide range of biotinylated antigens to ascertain if it is indeed as useful as I hope.

George P. Smith

NOTE ON COLLABORATION WITH ROBERT BIRD

Robert Bird of Genex Corporation intends to collaborate with me on the cloning of single-chain antibodies into fusion phage; he will supply the gene and will help characterize the binding properties of the phage. The University of Missouri and Genex are negotiating conditions of this collaboration, and as soon as this is done I will forward a letter of collaboration from Dr. Bird.

THE RESEARCH INSTITUTE OF SCRIPPS CLINIC

RICHARD A. LERNER
DIRECTOR

LITA ANNENBERG HAZEN PROFESSOR
OF IMMUNOCHEMISTRY

February 18, 1988

George P. Smith
Associate Professor
Division of Biological sciences
Tucker Hall
University of Missouri
Columbia, MO 65211

Dear Dr. Smith,

I was very interested in the idea of an "epitope library" and its potential use to answer questions about the nature of antigenic epitopes. It proposes a vehicle with which to extend in vivo some of our in vitro experiments with synthetic peptides. A drawback of peptide studies is that it is not feasible to synthesize all possible 6- or 7-residue peptides; an epitope library potentially overcomes this drawback by making it feasible to screen billions of sequences for binding to an antibody. This would be particularly useful for finding non-sequential immunogenic epitopes.

I will be glad to cooperate with your plan to use anti-MHr antibodies with the epitope library, once it is constructed. We will make available antibodies against the whole molecule and against synthetic peptides, as well as purified MHr.

My former research assistant, Hannah Alexander, now resides in Columbia, and has informed me of her intent to consult and collaborate with you in interpreting your results. She is intimately familiar with the MHr system, having done a large fraction of the crucial experiments and been an author on many of the major papers.

I am excited by these experiments, and am looking forward to a fruitful collaboration.

Sincerely,

Richard A. Lerner, M.D.

RAL/pmf

10666 North Torrey Pines Road La Jolla California 92037 (619) 457-9999

2414 Lynnwood Drive
Columbia, MO 65203

George P. Smith
Tucker Hall
University of Missouri
Columbia, MO 65211

February 25, 1988

Dear George,

I have read about your idea of an "epitope library," and I agree with you that the information it yields about antibody specificity might complement the things that have been learned with synthetic peptides. In particular, I think the point that your strategy allows the antibodies themselves to select the epitopes to study is well taken: it is only too obvious to those who work with synthetic peptides that only a tiny fraction of them can be synthesized and studied individually.

I would be delighted to collaborate with you on this project. Having myself done much of the work on anti-MHr antibodies in Richard's lab, and having been a co-author on three of the key papers (Tainer et al., Nature 312, 127-133, 1984; Geysen et al., Science 235, 1184-1190, 1987; Getzoff et al., Science 235, 1191-1196, 1987), I'm thoroughly familiar with all the reagents and procedures. My experience in analyzing the antigenic structure of MHr could help greatly in identifying features of the MHr surface that clones from your epitope library might be mimicking.

I'm excited by the prospects, and wish you luck in bringing them to reality.

Sincerely,

Hannah Alexander

Hannah Ben-Ze'ev Alexander

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*PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Smith, George P.

CHECKLIST

...N appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application. Do not attac... ...s page will be separately num... ...s page will not be duplicated ... ...be a part of the review process. It will be retained for PHS staff use only.

...F APPLICATION

... application (This ... ... is submitted to the PHS for the first time.)

X ... application ... GM 41478-01
... application replaces a ... ... version of a new, competing continuation or renewal application.)

... (COMPETING CONTINUATION) ...
(This application is to extend a ... grant beyond its current project period.)

... (SUPPLEMENT to grant ...
(This application is for additional ... to a previously awarded grant.)

... (CHANGE of principal investigator/program director.
Name of former principal investigator or program director: ...

... (RENEW application ... requested by the ... Department of State (DOS) and the country ...
... consent of the ... principal investigator/program director: ...

ASSURANCES (See GENERAL INFORMATION section of instructions.)

Rights to DHHS 441	Handicapped Individuals Form PHS 441	Sex Discrimination Form 639-A	Scientific Fraud (Misconduct) Assurance
[X] Filed	[X] Filed	[X] Filed	[X] Administrative review process has been estab... Reporting requirements of the published scie... misconduct regulations will be followed.
[ ] Not filed	[ ] Not filed	[ ] Not filed	

INDIRECT COSTS

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit o... ...rate established with the appropriate PHS Agency Cost Advisory Office. If the applicant organization is in the process of initially developing or renec... ...has established a rate with another federal agency, it should, immediately upon notification that an award will be made, develop a tentative ... proposal based on ... in accordance with the principles set forth in the pertinent *DHHS Guide for Esta...* ...cost rates, and submit it to the appropriate DHHS Regional Office or PHS Agency Cost Advisory Office. Indirect costs will *not* be paid on foreign ... ...truction grants, grants to Federal organizations, and grants to individuals, and usually not on conference grants. Follow any additional instructions c... ...Research Career Development Awards, ... National Research Service Awards, and the specialized grant applications listed in the GENERAL IN... ...section.

X DHHS Agreement Dated: Jan 21, 1987 [ ] No Indirect Costs Requ...

[ ] DHHS Agreement being negotiated with ... Regional Office.

[ ] No DHHS Agreement, but rate established with ... Date ...

NEGOTIATION*

... 12-month budget period:
Amount of base $ 94,798 x Rate applied 37.5% % = Indirect costs (a) $ 35,549

... proposed project period:
Amount of base $ 406,867 x Rate applied 37.5% = Indirect costs (b) $ 152,575

a) Add to total direct costs from page 4 and enter new total on FACE PAGE, Item 7b
b) Add to total direct costs from page 5 and enter new total on FACE PAGE, Item 8b

Check appropriate box(es):

[ ] Salary and wages base [X] Modified total direct costs base [ ] Other base (Explain below)

[ ] Off-site, other special rate, or more than one rate involved (Explain below)

Explanation (Attach separate sheet, if necessary.):

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE 5121MZ

NOTICE OF GRANT AWARD

DATE ISSUED: 06/08/90
GRANT NUMBER: 5 ROI GM41478-02

TYPE OF AWARD: RESEARCH
AUTHORIZED BY: 42 USC 241 42 CFR 52
AWARDED BY: NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

TOTAL PROJECT PERIOD: From 07/01/89 Through 06/30/

Title of Project or Area of Training
FILAMENTOUS FUSION PHAGE

Grantee Organization
CURATORS OF UNIV OF MISSOURI
OFFICE OF SPONSORED ADMIN
312 JESSE HALL
COLUMBIA, MO 65211

Principal Investigator/Program Director/Awardee
SMITH, GEORGE P PHD
UNIVERSITY OF MISSOURI
110 TUCKER HALL
COLUMBIA, MO 65211

APPROVED BUDGET
FOR BUDGET PERIOD 07/01/90 Through 06/30/91

Salaries and Wages	$ 37,838	
Fringe Benefits	8,336	
Total Personnel Costs		$ 46,174
Consultant Costs		
Equipment		
Supplies		21,104
Travel - Domestic		1,794
[illegible]		
[illegible] Care - Inpatient		
- Outpatient		
Alterations and Renovations		
Consortium/Contractual Costs		
Other		5,850
[illegible]		
Trainee Stipends		
Trainee Tuition and Fees		
Trainee Travel		
TOTAL DIRECT COSTS		$ 74,922

When PHS Prior Approval is required for rebudgeting, submit requests to Grants Management Official below.

AWARD COMPUTATION

DIRECT COSTS	$ 74,9
INDIRECT COSTS	$* 28,4
TOTAL	$ 103,3
Less Unobligated Balance [Prior Period(s)]	$
AMOUNT OF THIS AWARD →	$ 103,3

Base Dollars	x Rate Percentage	= Indirect Costs $
74,922	38.00	28,470

SUPPORT RECOMMENDED FOR REMAINDER OF PROJECT PERIOD*

Budget Period	Total Direct Costs (includes Stipends)
03	73,776
04	76,727
05	79,796
06	NONE

**Subject to availability of funds and satisfactory progress.

REMARKS
THIS GRANT IS INCLUDED UNDER EXPANDED AUTHORITIES (PHS CIRCULAR 89.02)
-SEE NIH GUIDE FOR GRANTS AND CONTRACTS, VOL. 18, NO. 36, 10/13/89.
SEE ATTACHED FOR ADDITIONAL TERMS AND CONDITIONS.

PROGRAM ADMINISTRATOR: DR. MARION ZATZ (301) 496-0334
#GRANTS MANAGEMENT: L ROBERTS/L CLARKE/M HAHN/M BUCK/G THOMPSON 301 496-7746

TERMS OF ACCEPTANCE: By acceptance of funds awarded under this grant, the grantee acknowledges that it will comply with terms and conditions in the following: (1) Legislation cited above; (2) Regulations cited above; (3) Special provisions noted above under remarks or attached to this notice; (4) 45 CFR Part 74 or 92, as applicable; (5) PHS Grants Administration Manual; (6) PHS Grants Policy Statement. The above order of precedence shall prevail.

ACCY—Common Accounting Number	CRS/Entity Identification No.	PHS List No./Object Class Code	Document Number
0-8423535		/41.4E	(08)R1GM41478A

PROGRAM OFFICIAL
W. Sue B. Shafer
W. SUE SHAFER, PH.D.
ASSOCIATE DIRECTOR FOR
PROGRAM ACTIVITIES
NAT. INST. GEN. MED. SCIENCES

PHS Grants Management Official
Carol L. Tippery
CAROL L. TIPPERY
GRANTS MANAGEMENT OFFICER
OFFICE ASSOC. DIRECTOR PROGRAM
ACTIVITIES, NIGMS

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Rev 7/88 [illegible]

ADDITIONAL TERMS AND CONDITIONS OF AWARD

Continuing Resolution:

This award is issued consistent with NIH and Institute policies and reflects, in part, the Continuing Resolution level with sequestration (Gramm/Rudman/Hollings Act).

SECTION 1 Rec'd 4-26-90

042

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Form approved through 3/31/91
OMB No. 0925-0001

REVIEW GROUP	TYPE	ACTIVITY	GRANT NUMBER (insert on all pages)
ALY		R01	GM41478-02

APPLICATION
FOR CONTINUATION GRANT

TOTAL PROJECT PERIOD
From: 07/01/89 Through: 06/30/94

REQUESTED BUDGET PERIOD
From: 07/01/90 Through: 06/30/91

To be verified by applicant. Check information in items 1 through 6. If incorrect, furnish correct information in item 13.

1. TITLE OF PROJECT
FILAMENTOUS FUSION PHAGE

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (name and address, street, city, state, zip code)
SMITH, GEORGE P
UNIVERSITY OF MISSOURI
TUCKER HALL
COLUMBIA, MO 65211

4. APPLICANT ORGANIZATION (name and address, street, city, state, zip code)
UNIVERSITY OF MISSOURI COLUMBIA
OFFICE OF SPONSORED ADMIN
305 JESSE HALL
COLUMBIA, MO 65211

5. ENTITY IDENTIFICATION NUMBER

2b. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
BIOLOGICAL SCIENCES

2c. MAJOR SUBDIVISION
COLLEGE OF ARTS AND SCIENCE

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR BIOMEDICAL RESEARCH SUPPORT GRANT (see instructions)
20 OTHER

6. TITLE AND ADDRESS OF OFFICIAL IN BUSINESS OFFICE OF APPLICANT ORGANIZATION
ASSISTANT DIRECTOR
UNIVERSITY OF MISSOURI COLUMBIA
OFFICE OF SPONSORED ADMIN
305 JESSE HALL
COLUMBIA, MO 65211

Complete the following (see instructions)

7. HUMAN SUBJECTS
7a. ☒ NO ☐ YES (☐ Exemption # OR ☐ IRB Approval Date)
7b. Assurance of Compliance #

8. VERTEBRATE ANIMALS
8a. ☒ NO ☐ YES ... IACUC Approval Date
8b. Animal Welfare Assurance #

9. PERFORMANCE SITE(S) (organizations and addresses)
405-406 Tucker Hall
University of Missouri
Columbia, MO 65211

10. COSTS REQUESTED FOR BUDGET PERIOD
10a. DIRECT $ 81,437 10b. TOTAL $ 112,383

11. INVENTIONS (see instructions)
☒ NO ☐ YES (☐ Previously reported OR ☐ Not previously reported)

TELEPHONE INFORMATION

	AREA CODE	TELEPHONE NO. AND EXTENSION
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Item 2a)	314	882-3344
12b. NAME OF BUSINESS OFFICIAL (Item 6) Ken Bittner, Asst. Director Office of Sponsored Program Administration	(314)	882-7560
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 15) Ken Bittner, Asst. Director Office of Sponsored Program Administration	(314)	882-7560

Proposal No. 9 011037-1

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWERS APPLY.
4. THE CURATORS OF THE
UNIVERSITY OF MISSOURI
312 Jesse Hall
5.
6. 312 Jesse Hall

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense. (U.S. Code, Title 18, Section 1001.)
SIGNATURE OF PERSON NAMED IN 2a (In ink. "Per" signature not acceptable)
DATE 4/20/90

15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)
SIGNATURE OF PERSON NAMED IN 12c (In ink. "Per" signature not acceptable)
Ken Bittner
DATE 4/24/90

PHS 2590 Optional (Rev. 10/88) PAGE 1

RETURN COMPLETED APPLICATION TO PHS AS SOON AS POSSIBLE: NO LATER THAN 1 MAY 1990

U.S. Department of Health and Human Services

Certification Regarding Lobbying

** The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

Award No.	Organizational Entity
5 R01 GM41478-03	THE CURATORS OF THE UNIVERSITY OF MISSOURI The University of Missouri - Columbia
Name and Title of Official Signing for Organizational Entity Ken Bittner, Asst. Director Office of Sponsored Program Administration	Telephone No. of Signing Official ( )882-7560
Signature of Above Official Ken Bittner	Date Signed 4/29/90

** Certification is based upon the provisions of the Interim Final Guidance, published in the Federal Register, December 20, 1989.

Proposal No. 9014033-1

REQUESTED BUDGET FOR NEXT BUDGET PER. (Follow instructions carefully)	FROM 7/1/90	THROUGH 6/30/.	GRANT NUMBER GM41478

ITEMIZE DIRECT COSTS REQUESTED FOR NEXT BUDGET PERIOD PERSONNEL (Applicant organization only) NAME	ROLE IN PROJECT	1 TYPE APPT.	2 % OF APPT.	3 EFFORT ON PROJ.	DOLLAR AMOUNT REQUESTED (Omit ce SALARY	FRINGE BENEFITS	TOTAL
George P. Smith	Principal Investigator			—	—	—	—
				—	12,720	3,180	15,90
Jamie K. Scott	Postdoctoral Fellow			—	—	—	—
Robert Davis	Research Specialist				23,320	5,830	29,15
Cindy Schopp	Laboratory Aide				5,088	51	5,13
				SUBTOTALS →	41,128	9,061	50,18

CONSULTANT COSTS (See instructions)

NONE

EQUIPMENT (Itemize)

NONE

SUPPLIES (Itemize by category) Isotopes 891; other specialty reagents 5,938; chemicals, ... electrophoresis supplies 2,375; small apparatus 2,227; pipette tips, microfuge tubes, microtiter plates, nitrocellulose and nylon membranes 2,078; petri dishes, media 1,484; labware, disposable culture tubes, office supplies, other general supplies 4,082; peptides 2,300; 8-channel pipetting device 450; replacement parts 1,114 — 22,93

TRAVEL	DOMESTIC	
	FOREIGN	1,95
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	

ALTERATIONS AND RENOVATIONS (Itemize by category)

CONSORTIUM/CONTRACTUAL COSTS (See instructions)

OTHER EXPENSES (Itemize by category) Postage $500; phone/FAX $690; publication and reproduction $550; repair $4,264; computer $355 — 6,359

TOTAL DIRECT COST (Enter on Page 1, Item 10a) → $ 81,437

8. SUPPLEMENTAL INFORMATION REGARDING *ITEMS* IN THE PROPOSED BUDGET FOR THE NEXT PERIOD WHICH REQUIRE EXPLANATION OR JUSTIFICATION *(SEE INSTRUCTIONS)*

A PLEA NOT TO CUT THIS BUDGET: The budget in my original proposal was, I believe, unusually frugal. In fact, extrapolated from 9 to 12 months, actual Supply costs will be $5,900 over the budgeted $13,600 and Other Expenses will be $1,364 over the budgeted $4,750, despite our efforts to be very conservative in spending. In order to accommodate these deficits as well as the overall 10% ($9,808) cut in the first year, I have had to forego my own 1989 summer salary ($9,000 plus $2,250 benefits) plus make an additional $4,670 worth of cuts in other categories. I hope not to have to make such severe accommodations in the second year.

PERSONNEL: No change. Besides my own summer salary, two essential personnel are budgeted: a Research Specialist (technician) and a Lab. Aide (dishwasher/media maker). No cuts in this category are feasible.

SUPPLIES: I have increased the projected actual supply expenses for the first year by 4% to partly compensate for inflation, and added two additional expenses: $450 for a multichannel pipettor, needed for the ELISA tests that have become a routine part of our characterization of clones; and $2,300 for peptides. Synthesis of a few free peptides is a crucial component of the "receptor project" described in the Progress Report Summary and would be extremely useful for several aspects of the orignally planned use of the Epitope Library for characterizing antibodies; we estimate that at least 30 peptides are needed altogether. The budgeted $2,300 would suffice for synthesis of 8-10 peptides in crude form by a commercial supplier (we would purify them ourselves); or for about 15 peptides if a central peptide synthesis facility that our campus is applying for comes to fruition. We hope to have collaborators synthesize the remaining peptides.

EQUIPMENT: I originally planned to replace one of the general-use ultracentrifuge rotors that our lab uses heavily. Although the need for this rotor has not diminished, I see no way it can be accommodated in this budget--certainly not if the budget is cut. We will continue to use the old rotors beyond their warranty dates, and try to find other sources of funding for replacements.

SECTION II CURRENT BUDGET PERI AND KEY PERSONNEL	FROM 7/1/89	THROUGH 6/30/90	GRANT NUMBER GM41478

The following pertains to your CURRENT PHS budget. This information will be used in determining the amount of support for the NEXT budget period.

A. CURRENT BUDGET	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (1)	ESTIMATED UNOBLIGATED BALANCE (2)	EXPLAIN ANY SIGNIFICANT ESTIMATED UNOBLIGATED BALANCE IN COLUMN 2 (3)
TOTAL DIRECT COSTS	88,279	0	
INDIRECT COSTS *(As provided)*	32,421	0	
TOTALS ——>	120,700	0	

B. CURRENT BUDGET PERIOD KEY PERSONNEL ENGAGED ON PROJECT *(Only if different)*

NAME, DEGREE(S) SSN	POSITION TITLE AND ROLE IN PROJECT DEPARTMENT AND ORGANIZATION	CHANGE IN % OF EFFORT
Smith, George P. Ph.D. Scott, Jamie K., M.D., Ph.D.	Postdoctoral Pellow, Dividion of Bioloqical Sciences and Department of Medicine, University of Missouri	

C. and D. *(Only if different)*
See instructions and provide the information required in items C. and D. Use this page and continuation pages as necessary.
C. Equipment: glass-fronted refrigerator $1475

SECTION III. PROPOSED KEY PERSONNEL FOR THE NEXT BUDGET PERIOD *(Only if different)*

NAME, DEGREE(S), SSN	POSITION TITLE AND ROLE IN PROJECT	DEPARTMENT AND ORGANIZATION
Scott, Jamie K. (see B above)		

OTHER SUPPORT
(Use continuation pages if necessary)

GRANT NUMBER
GM41478

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to delays in the award.

For each of the key personnel, list in separate groups: (1) *all* currently active support; (2) *all* applications and proposals pending review or funding. Include *all* Federal, non-Federal, and institutional research, training, and other grant, contract, and fellowship support at the applicant organization and elsewhere. If none, state "none." If no changes from the application that was the basis for this submission, state "no change."

For each item give: (a) the source of support, identifying number and title; (b) percentage of appointment on the project; (c) dates of entire project period; (d) annual direct costs; and (e) a brief description of the project for non-PHS supported projects. If part of a larger project, identify the principal investigator/program director and provide (a) above for the parent project and (b) through (e) for the subproject.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:
(1) CURRENTLY ACTIVE SUPPORT: (a)

NONE other than present grant (GM41478)

(2) NONE

Jamie K. Scott/Postdoctoral Fellow
(1) Currently active support
(a) Salary and fringe benefits supplied by present grant (GM41478)

(2) Applications pending review

SECTION IV
PROGRESS REPORT SUMMARY

GRANT NUMBER
GM41478

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR
George P. Smith

APPLICANT ORGANIZATION
UNIVERSITY OF MISSOURI

PERIOD COVERED BY THIS REPORT
FROM 7/1/90 THROUGH 4/20/90

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Filamentous Fusion Phage
(SEE INSTRUCTIONS)

See next page

PROGRESS REPORT SUMMARY

1. Plans for Second Year

In the summer of 1989, I had an unexpected opportunity: Dr. Jamie Scott applied to become a postdoctoral fellow in my laboratory. In order to take advantage of this opportunity, I gave up my research leave in order to pay her stipend for the year. Working together since late June, she and I have succeeded in constructing and characterizing the epitope library, as described below. She will continue to work in the second year, paid by an NRSA from NIH (her priority score virtually assures funding).

In the second year we will continue to use the epitope library to characterize antibody specificity as originally planned. Work is just now beginning toward constructing a library of "infectious antibodies," as planned in the original proposal; continuation of this work will be a major occupation of the second year. In addition, Dr. Scott will undertake a new initiative not originally envisioned: what we call the "receptor project."

Receptor Project. Three hormone receptors--those for epidermal growth factor, nerve growth factor, and interleukin-2--are being used in place of antibodies to "pan" the epitope library. We hope in this way to identify short peptides that bind tightly to the receptors. Selected peptides will be synthesized in free form and tested for their ability to agonize or antagonize the action of the hormones. Such peptides would be attractive candidates for drug development. If successful, this work would point the way to an entirely new approach to drug discovery, of great power and generality.

2. Research accomplished in the first year

We have constructed two epitope libraries, representing 2.3×10^7 and 2×10^8 primary clones. Theoretically these libraries display 21% and 79%, repectively, of the 64 million possible hexapeptides on the surface of infectious filamentous phage particles. Each phage displays a single hexapeptide, encoded by a single 18-bp oligonucleotide spliced into a coat protein gene.

Two monoclonal antibodies, both specific for the peptide epitope DFLEKI, have been used to affinity-purify phage from the 2.3×10^7-clone library, using the "biopanning" technique developed in my laboratory. The hopw was that only phage whose displayed hexapeptide binds antibody tightly would be isolated. The affinity-purified phage, which retain infectivity, were cloned and propagated by infecting E. coli, and their hexapeptides sequenced at the DNA level. Almost all the hexapeptides were very similar to DFLEKI, despite the fact that no information about the antibodies' specificity was incorporated into the library. This dramatically demonstrates the ability of the epitope library to find tight-binding peptide ligands for an antibody without prior knowledge of its specificity. It seems likely this technique can be extended to find peptide ligands for other, non-antibody ligates, such as hormone receptors; that is the basis of the receptor project outlined above.

The essential virtue of the epitope library is this: that a large and important part of the epitope universe can be encompassed in a few microliters of solution and effectively surveyed for specific affinity for an antibody, receptor or other ligate by simple recombinant DNA methods. Because specific binding is at the root of so many aspects of biology and medicine, there must be countless potential applications of this technology.

5. Publications

in press.

Submitted

Smith, George P.

CHECKLIST	GRANT NUMBER
	141478

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application. **Do not attach copies of this page to the duplicated copies of the application.**

ASSURANCES

The following certifications described below are made by checking the appropriate boxes and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application.

a. Delinquent Federal Debt. [X] No [ ] Yes (If "Yes," attach explanation.)

Before a grant award can be made, the applicant organization must certify that it is not delinquent on the repayment of any Federal debt. The certification applies to the applicant organization, not to the person signing the application as the authorized representative nor to the principal investigator/program director.

Examples of Federal debt include delinquent taxes, audit disallowances, guaranteed or direct student loans, FHA loans, business loans, and other miscellaneous administrative debts. For purposes of this certification, the following definitions of "delinquency" apply:

- For direct loans and fellowships (whether awarded directly to the applicant by the Federal Government or by an institution using Federal funds), a debt more than 31 days past due on a scheduled payment. (Definition excludes "service" payback under a National Research Service Award.)
- For guaranteed and insured loans, recipients of a loan guaranteed by the Federal Government that the Federal Government has repurchased from a lender because the borrower breached the loan agreement and is in default.
- For grants, organizations in receipt of a "Notice of Grants Cost Disallowance" which have not repaid the disallowed amount or which have not resolved the disallowance. (Definition excludes disallowances in an "appeal" status.)

Where the applicant discloses delinquency on debt to the Federal Government, the PHS shall (1) take such information into account when determining whether the prospective grantee organization is responsible with respect to that grant, and (2) consider not making the grant until payment is made or satisfactory arrangements are made with the agency to whom the debt is owed. Therefore, it may be necessary for the PHS to contact the applicant before a grant can be made to confirm the status of the debt and ascertain the payment arrangements for its liquidation. Applicants that fail to liquidate indebtedness to the Federal Government in a businesslike manner place themselves at risk of not receiving financial assistance from the PHS.

b. Debarment and Suspension. [X] No [ ] Yes (If "Yes," attach explanation.)

Before a grant award can be made, the applicant organization must certify, among other things, that neither it nor its principals are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency. Subawardees, that is, other corporations, partnerships, or other legal entities (called "lower tier" participants), must make the same certification to the applicant organization concerning their covered transactions. Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

c. Drug-Free Workplace. [X] Yes [ ] No (If "No," attach explanation.)

Before a grant award can be made, the applicant organization must certify that it will provide a drug-free workplace. The main points of the certification require the applicant organization to:

- Publish a statement notifying employees that the unlawful manufacture, distribution, dispensation, possession, or use of a controlled substance is prohibited in the workplace and specifying the actions that will be taken against employees for violation of such prohibition;
- Establish a drug-free awareness program;
- Require that each employee engaged in the performance of a grant or contract be provided a copy of the published statement;
- Notify the employee that as a condition of employment, the employee will abide by the terms of the statement;
- Notify the PHS awarding component of any employee convicted of a drug violation occurring in the workplace; and
- Require any employee who is convicted of a drug offense occurring in the workplace to participate in a rehabilitation program.

Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

INDIRECT COST CALCULATION

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

[X] DHHS Agreement Dated: December 12, 1988 [ ] No Indirect Costs Requested

[ ] No DHHS Agreement, but rates established with _____ DATE _____

*CALCULATION

Enter proposed budget period:

Amount of Base $ 81,437 x Rate Applied 38.0 % = Indirect Costs $ 30,946

Add to total direct costs from page 2 and enter new total on FACE PAGE, Item 10b

*Check appropriate box(es)

[ ] Salary and wage base [X] Modified total direct costs base [ ] Other base (Attach explanation)

[ ] Off-site, other special rate, or more than one rate involved (Attach explanation)

*This is the required last page of the application.

George P. Smith

PROGRESS REPORT SUMMARY

1. Plans for Third Year

Making epitope libraries more generally applicable. We will concentrate on two approaches toward the goal of being able to discover peptide ligands for almost any binding protein. First, we are constructing libraries to accommodate binding proteins that do not recognize short, linear peptides: libraries with longer randomized peptides; and "constrained" libraries, in which the randomized residues are displayed in the context of secondary structure imposed by disulfide bonds or non-covalent forces. Second, we are devising ways of surveying the libraries with binding proteins--especially monovalent binding proteins such as receptors--that bind the phage-borne ligand reversibly. We are especially hopeful of reversible, light-activated cross-linkers that would allow the researcher to "lock in" the bound state with a flash of light, thus gaining control over all the kinetic parameters of binding. The "receptor project" initiated in the second year will await progress on these fronts.

Antibodies as stand-ins for receptor. In some contexts it would be very useful to find peptide mimics of a non-peptide ligand whose natural receptor cannot be cloned or isolated in biochemically useful amounts. If a polyclonal antibody against the natural ligand is available, it may contain some component species that bind ligand in the same way as does the receptor. If, then, peptide ligands for such a polyclonal antibody are identified with the aid of the epitope library, some of the antibody-binding peptides may be agonists or antagonists of the natural, non-peptide ligand. We plan to test this concept with a number of well-characterized systems in which both the required polyclonal antibody and a simple assay for binding to the natural receptor are available. The ligand/receptor pairs include digoxin/Na,K-ATPase, biotin/avidin, cyclosporin/cyclophilin, and morphine/opioid receptor.

Antibody libraries. We will try to optimize a few monoclonal phage-antibodies for overall performance by repeated rounds of random mutation and affinity selection. This will validate the concept of improving affinity by an in vitro selection that mirrors affinity maturation in an immune animal; and potentially will also give us a well-adapted starting framework for randomizing the CDRs to generate a great diversity of specificities.

2. Research accomplished in the second year

Fusion-phage technology has gained considerable currency in the last year. We have to date filled 183 requests for "kits," consisting of the bacterial and phage strains and a 60-page instruction manual. A number of other groups have independently made technical advances, including most notably novel gene-III (Bass et al., Proteins: Structure, Function, and Genetics 8, 309-314, 1990) and gene-VIII fusions (Kang et al., PNAS, in press). We have distributed our hexapeptide library to about 50 groups, mostly academic; indeed, the primary library is now used up, and we are now

using an amplified library. We expect at least one company-- to make epitope libraries of various designs commercially available in the near future.

To date we have used 17 monoclonal anti-protein antibodies to survey our hexapeptide epitope library. Five were known in advance to be specific for linear epitopes--epitopes comprising 5-6 contiguous residues in the primary sequence of the antigen. All five identified ligands from the library that were very similar to the eliciting linear epitope; the phage-borne peptides all bound strongly to the antibody. Eleven of the monoclonals were known or thought in advance to be against assembled epitopes--epitopes comprising residues that are distant in the primary sequence of the antigen but contiguous in its native, folded structure. These eleven gave mixed results: Two identified peptide ligands related to a linear epitope on the antigen; the prior inference that these two antibodies are directed against assembled epitopes may have been wrong. Two identified mimotopes--peptides that bind the antibody (though weakly) but do not recognizably resemble any linear stretch of amino acids in the antigen's primary sequence. One identified a putative half-epitope--a peptide with weak similarity to a stretch of residues in the antigen that are thought to be part of an assembled epitope. The remaining six antibodies against assembled epitopes did not identify consensus peptides. One anti-protein monoclonal, whose epitope hadn't been characterized in advance, identified a peptide with very weak similarity to a region of the antigen primary sequence; the peptide may therefore be an example of either a mimotope or a half-epitope. When the library was surveyed with a monoclonal antibody against progesterone, a non-proteinaceous antigen, no consensus peptides could be found.

The library has also been surveyed with six polyclonal antibodies; the results are complicated by the heterogeneity of the antibody populations in this case, of course, but in general are consistent with the results with monoclonal antibodies.

Two major conclusions can be drawn from our results with antibodies. First, the uniform success with which antibodies against linear epitopes identify strong peptide ligands from the library--not only in our lab but in many others as well--shows that when the library displays strong ligands for an antibody, that antibody virtually always succeeds in identifying such ligands. Second, in light of the first conclusion we infer that our hexapeptide epitope library does not contain strong ligands for most antibodies against assembled or non-proteinaceous epitopes. At best, such antibodies identify weak mimotopes, but most do not identify any consensus peptide sequences. This implies, in turn, that if we are to achieve the goal of being able to identify strong peptide ligands for any binding protein, different library designs must be tried (see under Section 1 above).

Ten non-antibody binding proteins (insulin; receptors for IL-2, EGF and NGF; conconavilin A; protein A; the immunophilins FKBP and cyclophilin; HIV gp120; and the HIV receptor CD4) have been used to survey the library, with uniformly negative results--that is, no consensus sequences of peptide

ligands were identified. One possible explanation for this failure is that these proteins, like many antibodies, recognize epitopes that cannot be effectively mimicked by the short linear peptides displayed in our library. Another possibility, however, is that these proteins, being monovalent, bind their ligands reversibly, and therefore release bound phage during the prolonged washings of the biopanning procedure (see under Section 1 above).

Considerable progress has been made by many groups on "phage antibodies," the name now used (in place of our term "infectious antibodies") for filamentous phage displaying antibody domains on their surface. McCafferty et al. (Nature 348; 552-554, 1990) have already published a paper on their constructs, and I know of at least six other groups around the world, besides our own, who are actively pursuing this line of research (in press). We ourselves have constructed a phage displaying a single-chain anti-fluorescein antibody, exactly as planned in the original grant proposal. The phage can be enriched 5000-fold over wild-type phage by affinity-purification with proteins conjugated to fluorescein, a result comparable to the best from other labs. However, we find that high concentrations (20 μM) of free fluorescein hapten reduced binding by a factor of only about 50, suggesting that non-specific binding is about 100 fold higher than the background binding of wild-type phage. A similar level of non-specific binding---100 times the background with wild-type phage--was observed in two other negative controls: substitution of rhodamine- for fluorescein-conjugated protein in the affinity purification; and introduction of a single amino-acid substitution into the phage-borne antibody domain that abolishes binding without interfering with proper folding. It seems likely, therefore, that the foreign single-chain antibody domain displayed in our phage antibody is non-specifically "sticky" to some degree, possibly because it is malfolded some of the time or in some of the phage particles. For this reason, we do not consider wild-type phage, which do not display an antibody domain, as adequate negative controls for binding specificity (e.g., McCafferty et al.). Still, even though non-specific binding is higher than we would like, specific binding is much higher and we believe provides an adequate "handle" by which phage performance can be improved by mutation and selection (see Section 1 above).

5. Publications

Scott, J.K, and Smith, G.P. (1990) Search for peptide ligands with an epitope library. Science, 249, 386-390, 1990.

[CH]ECKLIST

GRANT NUMBER: 41478

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the applica[tion]. Do not attach copies of this page to the duplicated copies of the application.

ASSURANCES

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a. Delinquent Federal Debt. [X] No [ ] Yes (If "Yes," attach explanation.)

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- For guaranteed and insured loans, recipients of a loan guaranteed by the Federal Government that the Federal Government has repurchased from a lender because the borrower breached the loan agreement and is in default.
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b. Debarment and Suspension. [X] No [ ] Yes (If "Yes," attach explanation.)

Before a grant award can be made, the applicant organization must certify, among other things, that neither it nor its principals are presen[tly] debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency. Subawardees, that is, other corporations, partnerships, or other legal entities (called "lower tier" participants), mu[st] make the same certification to the applicant organization concerning their covered transactions. Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

c. Drug-Free Workplace. [X] Yes [ ] No (If "No," attach explanation.)

Before a grant award can be made, the applicant organization must certify that it will provide a drug-free workplace. The main points of t[he] certification require the applicant organization to:

- Publish a statement notifying employees that the unlawful manufacture, distribution, dispensation, possession, or use of a controlled substance is prohibited in the workplace and specifying the actions that will be taken against employees for violation of such prohibitio[n];
- Establish a drug-free awareness program;
- Require that each employee engaged in the performance of a grant or contract be provided a copy of the published statement;
- Notify the employee that as a condition of employment, the employee will abide by the terms of the statement;
- Notify the PHS awarding component of any employee convicted of a drug violation occurring in the workplace; and
- Require any employee who is convicted of a drug offense occurring in the workplace to participate in a rehabilitation program.

Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

INDIRECT COST CALCULATION

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

[X] DHHS Agreement Dated: 12/12/88 [ ] No Indirect Costs Requested

[ ] No DHHS Agreement, but rates established with ________ DATE ________

*CALCULATION

Enter proposed budget period:

Amount of Base $ 75,616 x Rate Applied 39 % = Indirect Costs $ 29,490

Add to total direct costs from page 2 and enter new total on FACE PAGE, Item 10b

*Check appropriate box(es)

[ ] Salary and wage base [X] Modified total direct costs base [ ] Other base (Attach explanation)

[ ] Off-site, other special rate, or more than one rate involved (Attach explanation)

PHS 2590 (Rev. 10/88) (Reprinted 9/89) PAGE 7*

*This is the required last page of the application.

REVISED

DEPARTMENT OF HEA[LTH] AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

5121MZ

NOTICE OF GRANT AWARD

OFFICIAL FILE COPY

[D]ATE ISSUED: 07/25/91

GRANT NUMBER: ALY
R01 GM41478-03

TYPE OF AWARD: RESEARCH

AUTHORIZED BY: 42 USC 241 42 CFR 52

TOTAL PROJECT PERIOD: From 07/01/89 Throu[gh] 06/30/9[?]

AWARDED BY: NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

Title of Project or Area of Training: FILAMENTOUS FUSION PHAGE

Grantee Organization:
CURATORS OF UNIV OF MISSOURI
OFFICE OF SPONSORED ADMIN
310 JESSE HALL
COLUMBIA, MO 65211

Principal Investigator/Program Director/Awardee:
SMITH, GEORGE P PHD
UNIVERSITY OF MISSOURI
110 TUCKER HALL
COLUMBIA, MO 65211

APPROVED BUDGET

FOR BUDGET PERIOD 07/01/91 Through 06/30/92

Salaries and Wages	$ 37,897	
Fringe Benefits	8,349	
Total Personnel Costs		$ 46,246
Consultant Costs		
Equipment		
Supplies		13,033
Travel - Domestic		1,533
- Foreign		
[Pat]ient Care - Inpatient		
- Outpatient		
Alterations and Renovations		
Consortium/Contractual Costs		
Other		4,552
Trainee Stipends		
Trainee Tuition and Fees		
Trainee Travel		
TOTAL DIRECT COSTS		$ 65,364

When PHS Prior Approval is required for rebudgeting, submit requests to Grants Management Official below.

AWARD COMPUTATION

DIRECT COSTS	$ 65,36[4]
INDIRECT COSTS	$* 25,49[2]
TOTAL	$ 90,85[6]
Less Unobligated Balance (Prior Period(s))	$
AMOUNT OF THIS AWARD	$ 90,85[6]

Base Dollars	x Rate Percentage	= Indirect Costs
65,364	39.00	25,492

SUPPORT RECOMMENDED FOR REMAINDER OF PROJECT PERIOD

Budget Period	Total Direct Costs (Includes Stipends)	Stipends
04	67,978	
05	70,697	
06	NONE	

**Subject to availability of funds and satisfactory progress.

REMARKS THE AMOUNT OF THIS AWARD REFLECTS AN INCREASE OF $654
THIS GRANT IS INCLUDED UNDER EXPANDED AUTHORITIES (PHS CIRCULAR 89.02)
-SEE NIH GUIDE FOR GRANTS AND CONTRACTS, VOL. 18, NO. 36, 10/13/89.

PROGRAM ADMINISTRATOR: DR. MARION ZATZ 301/496-0334.
GRANTS MANAGEMENT SPECIALIST: LINDA ROBERTS/GINNY THOMPSON 301-496-7746.

SEE ATTACHED FOR TERMS OF ACCEPTANCE AND ANY ADDITIONAL TERMS AND CONDITIONS.

[illegible]

[E]Y—Common Accounting Number	CRS/Entity Identification No.	PHS List No./Object Class Code	Document Number
1-8423535		/41.4E	(08)R1GM41478A

PROGRAM OFFICIAL
W. Sue Shafer
W. SUE SHAFER, PH.D.
ASSOCIATE DIRECTOR FOR
PROGRAM ACTIVITIES
NAT. INST. GEN. MED. SCIENCES

PHS Grants Management Official
[signature] for
CAROL L. TIPPERY
GRANTS MANAGEMENT OFFICER
OFFICE ASSOC. DIRECTOR PROGRAM
ACTIVITIES, NIGMS

PHS 1533 Copies distributed to Principal Investigator, Program Director or Awardee, and Business Office

Additional Terms and Conditions

Terms of acceptance: This grant is subject to the terms and conditions incorporated either directly or by reference in the following: (1) The grant program legislation cited on the first page; (2) The grant program regulations cited on the first page; (3) This award notice including terms and conditions, if any, noted below or attached to this notice; (4) PHS Grants Policy Statement including addenda in effect as of the beginning date of the budget period; (5) 45 CFR Part 74 or 92 as applicable. In the event there are conflicting or otherwise inconsistent policies applicable to the grant, the above order of precedence shall prevail. Acceptance of the grant terms and conditions is acknowledged by the grantee when funds are drawn or otherwise obtained from the grant payment system.

1. Any general program income accruing under this grant may be used in accordance with the additional costs alternative described in 45 CFR Part 74 Subpart F [74.42(e)].

2. This award is revised to reflect the correct indirect cost rate.

3. All previous terms and conditions of the original award issued on June 19, 1991 remain in effect.

SECTION 1 — 5/21MZ

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

APPLICATION FOR CONTINUATION GRANT

MAY 1 1991

REVIEW GROUP	TYPE	ACTIVITY	GRANT NUMBER (insert on all pages)
ALY	5	R01	GM41478-03

TOTAL PROJECT PERIOD
From: 07/01/89 Through: 06/30/94

REQUESTED BUDGET PERIOD
From: 07/01/91 Through: 06/30/92

To be verified by applicant. Check information in items 1 through 6. If incorrect, furnish correct information in item 13.

1. TITLE OF PROJECT
FILAMENTOUS FUSION PHAGE

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (name and address, street, city, state, zip code)
SMITH, GEORGE P
UNIVERSITY OF MISSOURI
110 TUCKER HALL
COLUMBIA, MO 65211

4. APPLICANT ORGANIZATION (name and address, street, city, state, zip code)
CURATORS OF UNIV OF MISSOURI
OFFICE OF SPONSORED ADMIN
312 JESSE HALL
COLUMBIA, MO 65211

5. ENTITY IDENTIFICATION NUMBER
1446000987C6

2b. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
BIOLOGICAL SCIENCES

2c. MAJOR SUBDIVISION
COLLEGE OF ARTS AND SCIENCE

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR BIOMEDICAL RESEARCH SUPPORT GRANT (see instructions)
20 OTHER

6. TITLE AND ADDRESS OF OFFICIAL IN BUSINESS OFFICE OF APPLICANT ORGANIZATION
ASSISTANT DIRECTOR
UNIVERSITY OF MISSOURI COLUMBIA
OFFICE OF SPONSORED ADMIN
312 JESSE HALL
COLUMBIA, MO 65211

Complete the following (see instructions)

7. HUMAN SUBJECTS
7a. [X] NO [ ] YES ([ ] Exemption # _____ OR [ ] IRB Approval Date _____)
7b. Assurance of Compliance # _____

8. VERTEBRATE ANIMALS
8a. [X] NO [ ] YES ... IACUC Approval Date _____
8b. Animal Welfare Assurance # _____

9. PERFORMANCE SITE(S) (organizations and addresses)
405-406 Tucker Hall
University of Missouri
Columbia, MO 65211

10. COSTS REQUESTED FOR BUDGET PERIOD
10a. DIRECT $ 75,616 10b. TOTAL $ 105,106

11. INVENTIONS (see instructions)
[X] NO [ ] YES ([ ] Previously reported OR [ ] Not previously reported)

TELEPHONE INFORMATION

	AREA CODE	TELEPHONE NO. AND EXTENSION
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Item 2a) George Smith	314	882-3344
12b. NAME OF BUSINESS OFFICIAL (Item 6) E. Kenneth Bittner	314	882-7560
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 15) Ken Bittner, Asst. Director Office of Sponsored Program Administration	(314)	882-7560

Proposal No. 911135-1

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWERS APPLY.
4) Curators of Univ of Missouri
Office of Sponsored Admin
310 Jesse Hall
Columbia, MO 65211

5) 43-600-3859

6) Assistant Director
University of Missouri Columbia
Office of Sponsored Admin
310 Jesse Hall
Columbia, MO 65211

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense. (U.S. Code, Title 18, Section 1001.)	SIGNATURE OF PERSON NAMED IN 2a (in ink. "Per" signature not acceptable) George P. Smith	DATE 4/29/91
15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)	SIGNATURE OF PERSON NAMED IN 12c (in ink. "Per" signature not acceptable) E. Kenneth Bittner	DATE 4/30/91

RETURN COMPLETED APPLICATION TO PHS AS SOON AS POSSIBLE!

U. S. Department of Health and Human Services

Certification Regarding Lobbying

** The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans and cooperative agreements) and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

George Smith

Award No.	Organizational Entity
5RD1 GM41478-03	THE CURATORS OF THE UNIVERSITY OF MISSC The University of Missouri - Columbia
Name and Title of Official Signing for Organizational Entity Ken Bittner, Asst. Director Office of Sponsored Program Administration	Telephone No. of Signing Official ((314) 882-7560
Signature of Above Official E. Kenneth Bittner	Date Signed 4/30/91

** Certification is based upon the provisions of the Interim Final Rule published in the Federal Register, February 26, 1990.

Proposal No. 9111135-1

REQUESTED BUDGET FOR NEXT BUDGET PERIOD Follow instructions carefully	FROM 7/1/91	THROUGH 6/30/92	GRANT NUMBER GM41478

A. ITEMIZE DIRECT COSTS REQUESTED FOR NEXT BUDGET PERIOD — DOLLAR AMOUNT REQUESTED (Omit cents)

PERSONNEL (Applicant organization only)

NAME	ROLE IN PROJECT	1 TYPE APPT.	2 % OF APPT.	3 EFFORT ON PROJ.	SALARY	FRINGE BENEFITS	TOTAL
George P. Smith	Principal Investigator				-	-	-
					15,000	1,200	16,200
Jamie K. Scott	Postdoctoral fellow				-	-	-
Robert Davis	Research Specialist				24,719	6,180	30,899
Xiaofen Gong	Research Specialist				-	-	-
Steve Haas	Lab. aide				6,000	60	6,060
	SUBTOTALS →				45,719	7,440	53,159

CONSULTANT COSTS (See Instructions)

none

EQUIPMENT (Itemize)

none

SUPPLIES (Itemize by category) isotopes $800; other specialty reagents $500; chemicals, electrophoresis supplies $2,000; small apparatus $1117; pipette tips, microtubes $2,500; petri dishes, media $1,400; other labware, office supplies, other general supplies $3,000; replacement parts $1,000 — 12,317

TRAVEL	DOMESTIC	2,500
	FOREIGN	
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	

ALTERATIONS AND RENOVATIONS (Itemize by category)

CONSORTIUM/CONTRACTUAL COSTS (See Instructions)

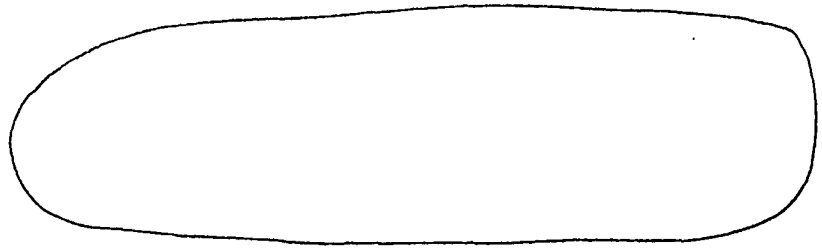
OTHER EXPENSES (Itemize by category) postage $1,000; phone/FAX $1,440; publication and reproduction $300; repairs $4,500; computer $400 — 7,640

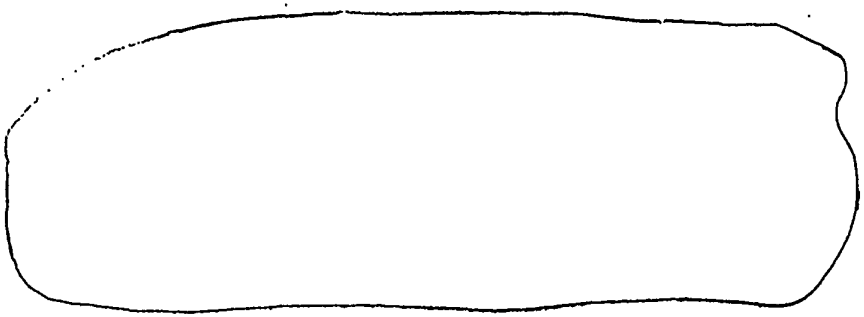
TOTAL DIRECT COST (Enter on Page 1, Item 10a) → $ 75,616

SECTION I (continued) NEXT BUDGET PERIOD	GRANT NUMBER GM41478

8. SUPPLEMENTAL INFORMATION REGARDING *ITEMS* IN THE PROPOSED BUDGET FOR THE NEXT PERIOD WHI REQUIRE EXPLANATION OR JUSTIFICATION. *(SEE INSTRUCTIONS)*

A NOTE ON THE BUDGET. The direct costs actually granted in the Second Year amounted to $78,713. This has been just adequate to meet the expenses of the lab because of two fortunate circumstances: (1) only $2,724 in direct costs were cut from the originally granted second-year budget; (2) the second-year budget originally included $10,000 to replace a common-equipment ultrcentrifuge rotor, which my Division purchased with other funds to free that money for my lab expenses. The direct costs budgeted here for the Third Year, even if they are not cut at all, are still $3,097 less than the expenses we will have incurred in the Second Year. Unfortunately, by frugal calculation I expect expenses in the Third Year to increase by -$7,000, not decrease, resulting in a total deficit of -$10,000. I have assigned all this deficit to the supply category, mostly because I hope to get collaborators to contribute supplies toward some joint projects; nevertheless, I don't expect such contributions to nearly make up the deficit.





SECTION II CURRENT BUDGET PER. AND KEY PERSONNEL	FROM 7/1/90	THROUGH 6/30/91	GRANT NUMBER GM41478

The following pertains to your CURRENT PHS budget. This information will be used in determining the amount of support for the budget period.

A. CURRENT BUDGET	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (1)	ESTIMATED UNOBLIGATED BALANCE (2)	EXPLAIN ANY SIGNIFICANT ESTIMATED UNOBLIGATED BALANCE IN COLUMN 2 (3)
TOTAL DIRECT COSTS	78,713	0	
INDIRECT COSTS *(As provided)*	29,911	0	
TOTALS →	108,624	0	

B. CURRENT BUDGET PERIOD KEY PERSONNEL ENGAGED ON PROJECT *(Only if different)*

NAME, DEGREE(S) SSN	POSITION TITLE AND ROLE IN PROJECT DEPARTMENT AND ORGANIZATION	CHANGE IN % OF EFFORT
Jamie K. Scott, M.D.,Ph.D.	Postdoctoral fellow. Division of Biological Sciences and Department of Medicine	

C. and D. *(Only if different)*
See instructions and provide the information required in items C. and D. Use this page and continuation pages as necessary.

SECTION III. PROPOSED KEY PERSONNEL FOR THE NEXT BUDGET PERIOD *(Only if different)*

NAME, DEGREE(S), SSN	POSITION TITLE AND ROLE IN PROJECT	DEPARTMENT AND ORGANIZATIO

OTH SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER GM41478

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to delays in the award. OTHER SUPPORT to be listed here refers to all current or requested support whether related to this application or not. If there are changes subsequent to submission, notify the Grants Management Official named on the Notice of Grant Award.

For each of the key personnel named on page 4, list, in three separate groups: (1) all currently active support; (2) all applications and proposals pending review or funding; and (3) applications and proposals planned or being prepared for submission. Include all Federal, non-Federal (e.g., for-profit, pharmaceutical, foundations), and institutional research, training, and other grant, contract, and fellowship support at the applicant organization and elsewhere. If part of a larger project, identify the principal investigator/program director and provide the data for both the parent project and the subproject. If none, state "none."

For each item give: (a) the source of support, identifying number and title; (b) percentage of appointment on the project; (c) dates of entire project period; (d) annual direct costs; (e) a brief description of the project; (f) whether the item overlaps, duplicates, or is being replaced or supplemented by the present application; delineate and justify the nature and extent of any scientific and/or budgetary overlaps or boundaries; and (g) any modifications that will be made should this continuation award be made.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:
(1) CURRENTLY ACTIVE SUPPORT: (a)

Present grant (GM41478)

Pending Support

(1) CURRENTLY ACTIVE SUPPORT

NIH NRSA 1F32GM13772, "Identifying mimetic peptides with an epitope library"; (b) 100%; (c) 7/1/90-6/30/92; (e)-(g) this covers Dr. Scott's salary plus $3,000 expenses for the "receptor project"; see NOTE ON THE PROTOS GRANT on p. 3.

(2)-(3) Dr. Scott anticipates submitting several proposals, but these would be for research to be conducted as an independent faculty member (assuming she is offered such a position), no longer affiliated with my lab.

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER GM41478	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR George P. Smith	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION University of Missouri-Columbia	7/1/90	6/30/91

TITLE OF PROJECT *(Repeat title shown in Item 1 on first page)*
Filamentous Fusion Phage

(SEE INSTRUCTIONS)

see next page

PROGRESS REPORT SUMMARY

1. Plans for Third Year

Making epitope libraries more generally applicable. We will concentrate on two approaches toward the goal of being able to discover peptide ligands for almost any binding protein. First, we are constructing libraries to accommodate binding proteins that do not recognize short, linear peptides: libraries with longer randomized peptides; and "constrained" libraries, in which the randomized residues are displayed in the context of secondary structure imposed by disulfide bonds or non-covalent forces. Second, we are devising ways of surveying the libraries with binding proteins--especially monovalent binding proteins such as receptors--that bind the phage-borne ligand reversibly. We are especially hopeful of reversible, light-activated cross-linkers that would allow the researcher to "lock in" the bound state with a flash of light, thus gaining control over all the kinetic parameters of binding. The "receptor project" initiated in the second year will await progress on these fronts.

Antibodies as stand-ins for receptor. In some contexts it would be very useful to find peptide mimics of a non-peptide ligand whose natural receptor cannot be cloned or isolated in biochemically useful amounts. If a polyclonal antibody against the natural ligand is available, it may contain some component species that bind ligand in the same way as does the receptor. If, then, peptide ligands for such a polyclonal antibody are identified with the aid of the epitope library, some of the antibody-binding peptides may be agonists or antagonists of the natural, non-peptide ligand. We plan to test this concept with a number of well-characterized systems in which both the required polyclonal antibody and a simple assay for binding to the natural receptor are available. The ligand/receptor pairs include digoxin/Na,K-ATPase, biotin/avidin, cyclosporin/cyclophilin, and morphine/opioid receptor.

Antibody libraries. We will try to optimize a few monoclonal phage-antibodies for overall performance by repeated rounds of random mutation and affinity selection. This will validate the concept of improving affinity by an in vitro selection that mirrors affinity maturation in an immune animal; and potentially will also give us a well-adapted starting framework for randomizing the CDRs to generate a great diversity of specificities.

2. Research accomplished in the second year

Fusion-phage technology has gained considerable currency in the last year. We have to date filled 183 requests for "kits," consisting of the bacterial and phage strains and a 60-page instruction manual. A number of other groups have independently made technical advances, including most notably novel gene-III (Bass et al., Proteins: Structure, Function, and Genetics 8, 309-314, 1990) and gene-VIII fusions (Kang et al., PNAS, in press). We have distributed our hexapeptide library to about 50 groups, mostly academic; indeed, the primary library is now used up, and we are now

using an amplified library. We expect at least one company- [redacted] to make epitope libraries of various designs commercially available in the near future.

To date we have used 17 monoclonal anti-protein antibodies to survey our hexapeptide epitope library. Five were known in advance to be specific for linear epitopes--epitopes comprising 5-6 contiguous residues in the primary sequence of the antigen. All five identified ligands from the library that were very similar to the eliciting linear epitope; the phage-borne peptides all bound strongly to the antibody. Eleven of the monoclonals were known or thought in advance to be against assembled epitopes--epitopes comprising residues that are distant in the primary sequence of the antigen but contiguous in its native, folded structure. These eleven gave mixed results: Two identified peptide ligands related to a linear epitope on the antigen; the prior inference that these two antibodies are directed against assembled epitopes may have been wrong. Two identified mimotopes--peptides that bind the antibody (though weakly) but do not recognizably resemble any linear stretch of amino acids in the antigen's primary sequence. One identified a putative half-epitope--a peptide with weak similarity to a stretch of residues in the antigen that are thought to be part of an assembled epitope. The remaining six antibodies against assembled epitopes did not identify consensus peptides. One anti-protein monoclonal, whose epitope hadn't been characterized in advance, identified a peptide with very weak similarity to a region of the antigen primary sequence; the peptide may therefore be an example of either a mimotope or a half-epitope. When the library was surveyed with a monoclonal antibody against progesterone, a non-proteinaceous antigen, no consensus peptides could be found.

The library has also been surveyed with six polyclonal antibodies; the results are complicated by the heterogeneity of the antibody populations in this case, of course, but in general are consistent with the results with monoclonal antibodies.

Two major conclusions can be drawn from our results with antibodies. First, the uniform success with which antibodies against linear epitopes identify strong peptide ligands from the library--not only in our lab but in many others as well--shows that when the library displays strong ligands for an antibody, that antibody virtually always succeeds in identifying such ligands. Second, in light of the first conclusion we infer that our hexapeptide epitope library does not contain strong ligands for most antibodies against assembled or non-proteinaceous epitopes. At best, such antibodies identify weak mimotopes, but most do not identify any consensus peptide sequences. This implies, in turn, that if we are to achieve the goal of being able to identify strong peptide ligands for any binding protein, different library designs must be tried (see under Section 1 above).

Ten non-antibody binding proteins (insulin; receptors for IL-2, EGF and NGF; conconavilin A; protein A; the immunophilins FKBP and cyclophilin; HIV gp120; and the HIV receptor CD4) have been used to survey the library, with uniformly negative results--that is, no consensus sequences of peptide

ligands were identified. One possible explanation for this failure is that these proteins, like many antibodies, recognize epitopes that cannot be effectively mimicked by the short linear peptides displayed in our library. Another possibility, however, is that these proteins, being monovalent, bind their ligands reversibly, and therefore release bound phage during the prolonged washings of the biopanning procedure (see under Section 1 above).

Considerable progress has been made by many groups on "phage antibodies," the name now used (in place of our term "infectious antibodies") for filamentous phage displaying antibody domains on their surface. McCafferty et al. (Nature 348; 552-554, 1990) have already published a paper on their constructs, and I know of at least six other groups around the world, besides our own, who are actively pursuing this line of research ([redacted] in press). We ourselves have constructed a phage displaying a single-chain anti-fluorescein antibody, exactly as planned in the original grant proposal. The phage can be enriched 5000-fold over wild-type phage by affinity-purification with proteins conjugated to fluorescein, a result comparable to the best from other labs. However, we find that high concentrations (20 μM) of free fluorescein hapten reduced binding by a factor of only about 50, suggesting that non-specific binding is about 100 fold higher than the background binding of wild-type phage. A similar level of non-specific binding---100 times the background with wild-type phage--was observed in two other negative controls: substitution of rhodamine- for fluorescein-conjugated protein in the affinity purification; and introduction of a single amino-acid substitution into the phage-borne antibody domain that abolishes binding without interfering with proper folding. It seems likely, therefore, that the foreign single-chain antibody domain displayed in our phage antibody is non-specifically "sticky" to some degree, possibly because it is malfolded some of the time or in some of the phage particles. For this reason, we do not consider wild-type phage, which do not display an antibody domain, as adequate negative controls for binding specificity (e.g., McCafferty et al.). Still, even though non-specific binding is higher than we would like, specific binding is much higher and we believe provides an adequate "handle" by which phage performance can be improved by mutation and selection (see Section 1 above).

5. Publications

Scott, J.K, and Smith, G.P. (1990) Search for peptide ligands with an epitope library. Science, 249, 386-390, 1990.

ECKLIST	GRANT NUMBER
	11478

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the app
Do not attach copies of this page to the duplicated copies of the application.

ASSURANCES

The following certifications described below are made by checking the appropriate boxes and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application.

a. Delinquent Federal Debt. [X] No [ ] Yes (If "Yes," attach explanation.)

Before a grant award can be made, the applicant organization must certify that it is not delinquent on the repayment of any Federal del
The certification applies to the applicant organization, not to the person signing the application as the authorized representative nor to
principal investigator/program director.

Examples of Federal debt include delinquent taxes, audit disallowances, guaranteed or direct student loans, FHA loans, business loans
other miscellaneous administrative debts. For purposes of this certification, the following definitions of "delinquency" apply:

- For direct loans and fellowships (whether awarded directly to the applicant by the Federal Government or by an institution using Fe
funds), a debt more than 31 days past due on a scheduled payment. (Definition excludes "service" payback under a National Res
Service Award.)
- For guaranteed and insured loans, recipients of a loan guaranteed by the Federal Government that the Federal Government has
repurchased from a lender because the borrower breached the loan agreement and is in default.
- For grants, organizations in receipt of a "Notice of Grants Cost Disallowance" which have not repaid the disallowed amount or whic
have not resolved the disallowance. (Definition excludes disallowances in an "appeal" status.)

Where the applicant discloses delinquency on debt to the Federal Government, the PHS shall (1) take such information into account wl
determining whether the prospective grantee organization is responsible with respect to that grant, and (2) consider not making the gra
until payment is made or satisfactory arrangements are made with the agency to whom the debt is owed. Therefore, it may be necessa
for the PHS to contact the applicant before a grant can be made to confirm the status of the debt and ascertain the payment arrangeme
for its liquidation. Applicants that fail to liquidate indebtedness to the Federal Government in a businesslike manner place themselves a
risk of not receiving financial assistance from the PHS.

b. Debarment and Suspension. [X] No [ ] Yes (If "Yes," attach explanation.)

Before a grant award can be made, the applicant organization must certify, among other things, that neither it nor its principals are prese
debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal
department or agency. Subawardees, that is, other corporations, partnerships, or other legal entities (called "lower tier" participants), m
make the same certification to the applicant organization concerning their covered transactions. Please refer to the pertinent DHHS
implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

c. Drug-Free Workplace. [X] Yes [ ] No (If "No," attach explanation.)

Before a grant award can be made, the applicant organization must certify that it will provide a drug-free workplace. The main points of t
certification require the applicant organization to:

- Publish a statement notifying employees that the unlawful manufacture, distribution, dispensation, possession, or use of a controlled
substance is prohibited in the workplace and specifying the actions that will be taken against employees for violation of such prohibit
- Establish a drug-free awareness program;
- Require that each employee engaged in the performance of a grant or contract be provided a copy of the published statement;
- Notify the employee that as a condition of employment, the employee will abide by the terms of the statement;
- Notify the PHS awarding component of any employee convicted of a drug violation occurring in the workplace; and
- Require any employee who is convicted of a drug offense occurring in the workplace to participate in a rehabilitation program.

Please refer to the pertinent DHHS implementing regulations, Title 45 Code of Federal Regulations Part 76, for complete certification requirements.

INDIRECT COST CALCULATION

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

[X] DHHS Agreement Dated: 12/12/88 [ ] No Indirect Costs Requested

[ ] No DHHS Agreement, but rates established with _____ DATE _____

*CALCULATION

Enter proposed budget period:

Amount of Base $ 75,616 x Rate Applied 39 % = Indirect Costs $ 29,490

Add to total direct costs from page 2 and enter new total on FACE PAGE, Item 10b

*Check appropriate box(es)

[ ] Salary and wage base [X] Modified total direct costs base [ ] Other base (Attach explanation)

[ ] Off-site, other special rate, or more than one rate involved (Attach explanation)